VOLUME 42 NOVEMBER, 1952 NUMBER 11

# Phytopathology

## An International Journal

DEC 10 1952
SCIENCE LIBRARY

*Official Organ of*
THE AMERICAN PHYTOPATHOLOGICAL SOCIETY

## CONTENTS

The retarding effect of certain plant hormones on tobacco mosaic symptoms
CARL W. NICHOLS 579

The influence of pH and zinc on vesicle formation in Puccinia coronata avenae corda
EUGENE L. SHARP AND FREDERICK G. SMITH 581

Sulfur soil treatment for control of sweet potato soil rot incited by Streptomyces ipomoea
W. J. HOOKER AND LEWIS E. PETERSON 583

Studies on internal browning of tomato — S. P. RAYCHAUDHURI 591

A new Sclerospora on Dichanthium annulatum
M. J. THIRUMALACHAR AND M. J. NARASIMHAN 596

Examination of the possibility of therapy of plant disease with ionizing radiation
PAUL E. WAGGONER AND A. E. DIMOND 599

Adaxial crack stem of celery
JOHN T. MIDDLETON, BERNARR J. HALL, R. T. WEDDING AND J. B. KENDRICK, JR. 603

Blights of snap bean in Georgia — E. S. LUTTRELL AND K. H. GARREN 607

Antibiotics and antagonistic microorganisms as control agents against damping-off of alfalfa — K. E. GREGORY, O. N. ALLEN, A. J. RIKER AND W. H. PETERSON 613

Rod-shaped virus of radish mosaic — WILLIAM N. TAKAHASHI 623

Effect of temperature on the development of internal cork lesions in sweet potato roots
L. W. NIELSEN 625

Inoculation techniques for evaluating resistance to Pseudomonas solanacearum
N. N. WINSTEAD AND ARTHUR KELMAN 628

Phytopathological notes:
Ephelis on Echinochloa crus-galli Beauv. and Setaria italica Beauv.
N. S. VENKATAKRISHNIAH 634

Tilletia elymi, an apparently homothallic species — C. S. HOLTON 635

NEWS

Subscription, $10.00 per year

Office of Publication—Monumental Printing Company, Baltimore, Md.

Entered as second-class matter at the post office at Baltimore, Md.

# *Du Pont Announces*

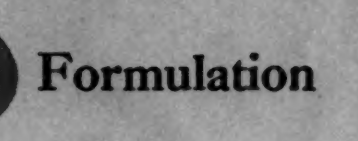
# ARASAN® SF-X

## Seed Disinfectant

## New DUSTLESS Formulation

**No Dust** in treating or bagging

**No Dust** in storage or shipment



**No Dust** in planting

**This new formulation** of "Arasan"—dustless "Arasan" SF-X—provides new ease and convenience in treating seed, and in handling and merchandising your seed. This exceptional new formulation eliminates the dust problem all the way to the man who plants the seed. Sticks tight to seed, gives exceptional protection against disease.

It's ideal for vegetables, corn, soybeans, grass, legumes and sorghum. "Arasan" SF-X replaces "Arasan" SF for the slurry method of treating, and you can also use it dry in seed mixers or blenders for treating grass and small-seeded legumes. One pound of "Arasan" SF-X treats 300 lbs. of seed.

*On all chemicals always follow directions for application. Where warning or caution statements on use of the product are given, read them carefully.*

DU PONT
REG. U.S. PAT. OFF.

BETTER THINGS FOR BETTER LIVING
...THROUGH CHEMISTRY

**ARASAN® SF-X**

SEED DISINFECTANT AND PROTECTANT

When answering our advertisements mention Phytopathology.

# THE RETARDING EFFECT OF CERTAIN PLANT HORMONES ON TOBACCO MOSAIC SYMPTOMS [1]

Carl W. Nichols

It has been reported that naphthaleneacetic acid (1, 3) and indolebutyric acid (2) inhibit multiplication of tobacco mosaic virus (TMV) in tobacco stem tissue cultures. The purpose of this paper is to report the effect of these plant hormones on symptoms produced by TMV when the compounds are applied to growing plants in the greenhouse. The effects of different concentrations of the hormones on plant growth are also recorded, since treatments that produce severe injury would have no use as control measures.

METHODS.—The hormones were applied to young tobacco plants in the greenhouse by spraying with an atomizer, by adding them to the soil in which the plants were growing, or by both methods. When plants were sprayed with the hormones, they were watered with distilled water (DW). When plants were watered with the hormones, they were sprayed with distilled water. Check plants were sprayed and watered with distilled water. In most cases the hormones were applied once a day. Application of hormones was started 1 or 2 days before the plants were inoculated with TMV. Plants were inoculated by applying the juice from macerated leaves to 1 leaf on each plant with a stiff brush; the inoculated leaf was immediately washed. The number of days after inoculation when tobacco mosaic symptoms appeared (incubation period) was used as a criterion of the retardation of symptoms.

RESULTS.—Tobacco plants were sprayed, watered, or sprayed and watered every day with naphthaleneacetic acid (NAA) in concentrations of 1 and 10 mg./l. Only the latter concentration caused any retardation in development of tobacco mosaic symptoms. Spraying with naphthaleneacetic acid at this concentration increased the average incubation period from 7.3 to 15.5 days. In 1 typical test, where 3 plants were used per treatment, the incubation periods were as follows: plants sprayed and watered with naphthaleneacetic acid, 16 days; plants sprayed with the hormone, 13-14 days; plants watered with the hormone, and nontreated checks, developed symptoms in 6 days. Two additional tests produced similar results. When the retarded symptoms occurred on plants that were sprayed with naphthaleneacetic acid at this concentration, they were never so severe as in the checks; also, typical vein-clearing usually did not appear. Naphthaleneacetic acid-sprayed plants showed marked effects due to the hormone. Plants were severely stunted and many blister-like swellings occurred on stems and petioles. Leaves were distorted and their margins rolled downward. Accordingly, no further tests were made with this compound.

Kutsky (2) reported that indolebutyric acid (IBA) in concentrations of 10 and 100 mg./l. reduced the concentration of TMV in tobacco stem tissue cultures. In studies reported here tobacco plants were treated every day with this compound at concentrations of 0.1, 1.0, 10, and 100 mg./l. Only the concentration of 100 mg./l. caused any retardation of tobacco mosaic symptoms. In plants sprayed with this concentration, the incubation period for symptoms was from 13 to more than 20 days, as compared with an average of 6 days in the checks.

There appeared to be a slight difference in the growth habit of the tobacco plants sprayed with indolebutyric acid as compared with those sprayed with distilled water. Hormone-sprayed plants were more upright, with younger leaves pointing straight up or upward at an acute angle to the main stem. Margins of older leaves had a slight tendency to roll downward. Sometimes younger leaves were somewhat twisted. A few hormone-sprayed plants developed blister-like swellings on the stems. All hormone-sprayed plants developed a darker green color than check plants. The typical early vein-clearing symptom of tobacco mosaic did not appear on any of the plants that were sprayed with indolebutyric acid. The first disease symptom to appear was a few dark green areas on medium and small-sized leaves, in many cases, along the lateral and midveins of the smaller leaves. At no time was the mottling so severe on the indolebutyric acid-sprayed plants as on the check plants.

A test was set up to determine whether there was any difference in the fresh weight of indolebutyric-acid-sprayed and distilled-water-sprayed plants. Twenty-four tobacco plants were divided into 4 groups of 6 each. The division was so made that the size range in the plants was distributed as evenly as possible among the 4 groups. Two of the groups were sprayed once a day with indolebutyric acid at a concentration of 100 mg./l. The other 2 groups were sprayed each day with distilled water. All the plants in 1 of the indolebutyric acid groups and in 1 of the distilled-water-sprayed groups were inoculated on the second day with TMV. Twenty days after inoculation the fresh weight was obtained for the top of each plant. Plants sprayed with indolebutyric acid and inoculated with TMV had an average fresh weight of 37.8 gm.; those sprayed with indolebutyric acid and noninoculated had an average fresh weight of 37.4 gm.; plants sprayed with distilled water and inoculated had an average fresh weight of 31.6 gm.; those sprayed with distilled water and noninoculated had an average fresh weight of 34.6 gm.

Although the small number of plants involved and

[1] Accepted for publication June 14, 1952.

The writer wishes to express his appreciation to T. E. Rawlins for his advice and criticism throughout the work reported in this paper.

TABLE 1.—*Effect of indolebutyric acid (500 mg./l.) applied to tobacco plants on tobacco mosaic symptoms*

| Treatment | Days after inoculation when symptoms appeared | | |
|---|---|---|---|
| | Plant number | | |
| | 1 | 2 | 3 |
| Sprayed every day with IBA | [a] | [a] | [a] |
| Sprayed every 2 days with IBA | [a] | 21 | 19 |
| Sprayed every 3 days with IBA | 20 | 16 | 19 |
| Sprayed every 4 days with IBA | 21 | [a] | 15 |
| Sprayed every 5 days with IBA | [a] | 17 | 21 |
| Sprayed every 6 days with IBA | [a] | 19 | 16 |
| Sprayed every 7 days with IBA | 20 | 20 | 17 |
| Sprayed every 8 days with IBA | [a] | 14 | 15 |
| Sprayed every 9 days with IBA | [a] | 20 | 15 |
| Sprayed every day with DW (check) | 5 | 5 | 5 |

[a] Symptoms absent 23 days after inoculation.

the variability in the individual plant weights did not allow definite conclusions to be drawn, it appeared that spraying with indolebutyric acid did not reduce the total fresh weight of the normal or diseased plants.

The possibility that tobacco plants could tolerate a higher concentration of indolebutyric acid was investigated. Tobacco plants were sprayed with indolebutyric acid at a concentration of 500 mg./l. at intervals varying from every day to every ninth day. The plants were inoculated with TMV on the second day. On the days when they were not sprayed with the hormone, the plants were sprayed with distilled water. All the hormone-sprayed plants developed marked downward cupping of the leaves within a few hours of the first application. Most of these plants made some degree of recovery from this cupping condition and, when the experiment was terminated after 23 days, the effect of the hormone varied with the number of applications. The plants that were sprayed every day were stunted, and had necrotic areas on the older leaves and severe downward rolling of the leaf margins. This was also true to a slightly lesser extent for the plants that were sprayed every second day. The plants that were sprayed every third or fourth day showed considerable downward rolling of the leaf margins and some stunting. In the other treated plants, the upright appearance of the younger leaves and the slight downward rolling of the older leaves, reported earlier, was apparent. No stunting was evident. All the treated plants showed blister-like swellings on stems and petioles, the plants sprayed every day being most severely affected.

The effect of these treatments on retardation of symptoms is shown in Table 1. All the treatments prolonged the time required for symptom expression; all but the first appeared equally effective. However, spraying the plants every day to every 4 days caused such severe injury as to make the treatments impractical as control measures. Spraying every 5 to 9 days retarded the appearance of symptoms, decreased their severity, and did not visibly stunt the plants.

In order to determine whether the symptomless plants in the experiment reported in Table 1 were carrying TMV, they were allowed to grow without spraying until they developed symptoms. All these plants developed symptoms within 7 days of the cessation of spraying.

DISCUSSION.—Results show clearly that indolebutyric acid is more effective than naphthaleneacetic acid in the retardation and inhibition of tobacco-mosaic symptoms. The indolebutyric acid is superior in that certain concentrations cause a distinct retardation in the development of symptoms and a decrease in their severity, without causing any detectable stunting of the host. Naphthaleneacetic acid causes a severe stunting of the host when sufficient hormone to cause a marked retardation of symptoms is applied. No concentration of either hormone was found to prevent all symptoms of tobacco mosaic in tobacco without causing injury to the tobacco plant.

Results do not show how much of the effect of the hormone on mosaic symptoms is due to decreased virus concentration in the host. The fact that others (1, 2, 3) have found these hormones to decrease TMV in tissue cultures suggests that decreased virus concentration may be one of the factors responsible for retardation and decreased severity of symptoms.

## SUMMARY

Spraying tobacco plants with naphthalene acetic acid or indolebutyric acid solutions retarded development of symptoms and decreased severity of tobacco mosaic symptoms. Indolebutyric acid was superior in that at certain concentrations it produced these effects without stunting the host. Best results were obtained by spraying daily with 100 mg./l. or at intervals of 5 to 9 days with 500 mg./l. No concentration of either hormone was found to prevent all mosaic symptoms without causing injury to the tobacco plant.

DEPARTMENT OF PLANT PATHOLOGY
UNIVERSITY OF CALIFORNIA
BERKELEY, CALIFORNIA

## LITERATURE CITED

1. AUGIER DE MONTGREMIER, H., AND G. MOREL. 1948. Sur la diminution de la teneur en virus (Marmor tabaci Holmes) de tissus de tabac cultivés in vitro. Compt. Rend. **227**: 688-689.
2. KUTSKY, R. 1952. Effects of indolebutyric acid and other compounds on virus concentration in plant tissue cultures. Science **115**: 19-20.
3. KUTSKY, R. J., AND T. E. RAWLINS. 1950. Inhibition of virus multiplication by naphthalene acetic acid in tobacco tissue cultures as revealed by a spectrophotometric method. Jour. Bact. **60**: 763-766.

# THE INFLUENCE OF pH AND ZINC ON VESICLE FORMATION IN PUCCINIA CORONATA AVENAE CORDA [1]

Eugene L. Sharp and Frederick G. Smith

Uredospore development of cereal rusts is known primarily through the classical histological work of Pole-Evans (6). He described and illustrated the development of a number of *Puccinia* species on the host, including *P. coronata*, and found that development in each species followed a characteristic pattern. Differences in appressoria, substomatal vesicles and infection hyphae were the distinguishing features. Ruttle and Fraser (7) studied the development of *P. coronata* both on a susceptible and a resistant variety of oats and obtained similar results. Hurd Karrer and Rodenhiser (4) were the first to report the formation of structures corresponding to appressoria, substomatal vesicles and infection hyphae on nutrient media, hereafter referred to as "vesicle formation." These vesicles occurred very rarely (less than 1 in a 1000) and the factors affecting their formation were unknown, although they were never observed on plain water agar. Dickinson, (1) working with specially prepared membranes, reported analogous structures with several *Puccinia* species and attributed their formation to a contact stimulus. Attempts to prolong uredospore growth by the addition of growth substances to artificial media have been unsuccessful (3).

In the present work, *P. coronata* has given as high as 40 per cent vesicle formation when 3 per cent gelatin was used as substrate with the proper pH and zinc ($ZnSO_4$) concentration. Figure 1 shows examples of these structures which conform very closely to those described in serial leaf sections (6, 7). Some of the main features are: (a) the shape of appressoria, (b) the shape of substomatal vesicles and the position of septa, and (c) the size and arrangement of the infection hyphae. The appressoria were variable in shape and smooth in outline but frequently had many finger-like projections as described by Ruttle and Fraser. In all cases, there was 1 septum across the substomatal vesicle which was always somewhat off-center. These septa could be readily observed after the orange pigment bands had passed out into the infection hyphae after about 3 days. At this time the germ tube and often the appressoria collapsed leaving only the substomatal vesicle and the infection hyphae in an expanded condition. The infection hyphae often branched or formed lobe-like projections which were rapidly filled with the pigment.

The effect of pH and zinc concentration on vesicle formation of 1 lot of spores is shown in Figure 2. In the pH range 6.2 to 6.6, vesicle formation appeared at about 6 p.p.m. of zinc, reached a peak between 9 and 14 p.p.m. and was not observed at 18 p.p.m. or above. At 14 p.p.m. the first signs of toxicity appeared, mainly as shortening of the germ tube. At 18 and 23 p.p.m. the germ tubes were markedly decreased in length and were frequently aborted at the higher concentration. The percentage of spores germinating was also somewhat lower at the 2 highest zinc concentrations. At pH 6.9 a few vesicles were formed at 18 p.p.m. indicating a somewhat higher optimum zinc concentration at the upper end of the pH range. At pH 5.9 and 7.1 no vesicles were observed. The spore lot illustrated in Figure 2 was a greenhouse collection which had been stored about 5 months (5-10° C.) at the time of the tests and was mainly a mixture of races 45 and 57. Other spore lots, including recent collections, have shown the same general pattern but the percentage of vesicles formed has varied from 15 to 40.

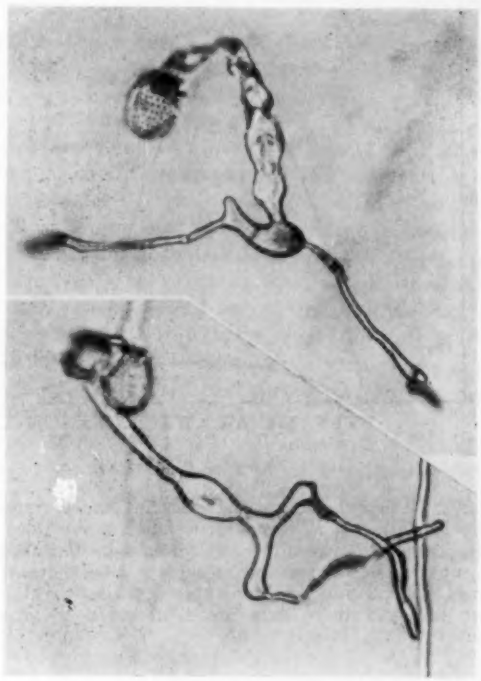


FIG. 1.—The development of appressoria, substomatal vesicles and infection hyphae in *P. coronata* on a zinc-gelatin substrate.

Zinc and pH also influenced a particular type of branching of the germ tube in which a secondary branch was formed close to the uredospore and was usually much shorter than the main branch. This branching effect occurred in a range of pH and zinc concentration corresponding very closely to that of vesicle formation but extending to a somewhat higher

[1] Accepted for publication June 23, 1952.

Journal Paper No. J-2082 of the Iowa Agricultural Experiment Station, Ames, Iowa. Project No. 1088. These studies were aided by a contract between the Office of Naval Research, Department of the Navy and Iowa State College (NR 134-109).

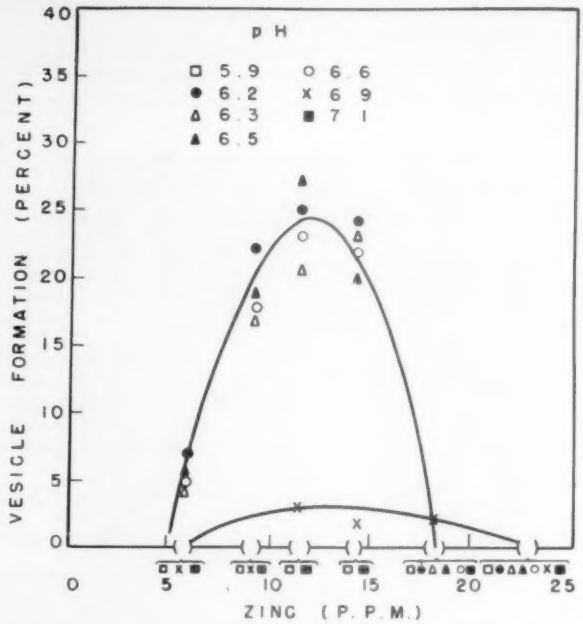



FIG. 2.—The influence of pH and zinc concentration on vesicle formation in *P. coronata*. Symbols below the brackets indicate 0 per cent vesicle formation. Variations in per cent of vesicle formation at a given zinc concentration between pH 6.2 and 6.6 were not significant by the chi-square test and these data are represented by a single curve.

pH. This type of branching, as well as vesicle formation, did not occur at pH 5.9 with any zinc concentration. Over the optimum pH range, however, there was a small amount of branching (5 per cent) with no zinc which was increased to 50 per cent with zinc addition.

Two per cent water agar at the optimum pH and with zinc concentrations up to 90 p.p.m. (where toxicity became apparent) gave no vesicles. However, on 0.5 per cent agar a few vesicles (5 per cent) were observed at 11 and 23 p.p.m. of zinc and branching was prevalent. Most of the vesicles were slow in appearing (70 hours) and were not typical in that the appressorium was not present and the substomatal vesicle was closely appressed to the spore.

Iron ($FeCl_3$), copper ($CuSO_4$), manganese ($MnSO_4$) and molybdenum ($(NH_4)_2\ MoO_4$) were substituted for zinc up to the range of toxicity with negative results. Calcium ($CaCl_2$) and magnesium ($MgCl_2$) also had no effect on the germ tube up through 100 p.p.m. The concentrations at which toxicity was observed for these ions generally were higher than that for zinc.

It appears that zinc has some specific effect on the germ tubes similar to that observed when they come in contact with the guard cells of the host. Zinc is known to have a marked effect on the metabolism of several fungi (2). It is essential in the utilization of intermediary metabolites in cell synthesis and functions in enzyme systems involved in carbohydrate and protein metabolism. Zinc also has been reported to increase vacuolization in fungus hyphae (5). This vacuolization was reported by Ruttle and Fraser (7) to precede the formation of the finger-like projections in the appressoria of *P. coronata* in contact with guard cells. Such vacuolization, preceded by branching, also has been observed in the present work, both in the case of the appressoria and secondary branching adjacent to the uredospore. Further work is under way to determine whether the gelatin as well as the zinc has a specific role in vesicle formation either through its physical or chemical properties. Further work also should determine if other species show a similar zinc response. Results of this investigation may provide a new technic for distinguishing closely related forms of *Puccinia* species on the basis of vesicle structure. Finally, the zinc response seems to open new possibilities in nutritional studies with cereal rusts.

DEPARTMENT OF BOTANY AND PLANT PATHOLOGY
IOWA STATE COLLEGE, AMES, IOWA.

## LITERATURE CITED

1. DICKINSON, SYDNEY. 1949. Studies in the physiology of obligate parasitism. II The behavior of the germ-tubes of certain rusts in contact with various membranes. Ann. Bot. **13**: 219-236.
2. FOSTER, J. W. 1949. Chemical activities of fungi. Academic Press Inc. New York, New York.
3. GOTTLIEB, DAVID, AND HELEN HART. 1943. Growth substances and the rust fungi. Phytopathology. **33**: 724-728.
4. HURD-KARRER, A. M., AND H. A. RODENHISER. 1947. Structures corresponding to appressoria and substomatal vesicles produced on nutrient-solution agar by cereal rusts. Amer. Jour. Bot. **34**: 377-384.
5. IWANOFF, K. S. 1904. Ueber die Wirkung einiger Metallsalze und einatomiger Alkohole auf die Entwickelung von Schimmelpilzen. Centralblatt Bakt. Parasitenk. Abt. II. **13**: 139-144.
6. POLE-EVANS, I. B. 1907. The cereal rusts. I. The development of their uredo mycelia. Ann. Bot. **21**: 441-466.
7. RUTTLE, MABEL L. AND W. P. FRASER, 1927. A cytological study of *Puccinia coronata* Cda. on Banner and Cowra 35 oats. Publ. in Bot. Calif. Univ. **14**: 21-72.

# SULFUR SOIL TREATMENT FOR CONTROL OF SWEET POTATO SOIL ROT INCITED BY STREPTOMYCES IPOMOEA [1]

W. J. Hooker and Lewis E. Peterson [2]

Sweet potato soil rot, soil pox, ground rot, pit, or pox incited by *Streptomyces ipomoea* (Person and Martin) Waksman and Henrici has gradually become more important during the past 30 years. At present it is the major disease of the crop in the sandy soils of southeastern Iowa. Soil rot is most widespread in the Buckner coarse sand-soil near Muscatine, but recently has become more prevalent in the Buckner loamy sandy soil around Conesville, Iowa, approximately 30 miles distant. Severely infested fields are generally abandoned for further sweet potato culture.

Soil rot causes blackened, relatively deep, firm lesions on the fleshy roots. Enlargement of roots is generally restricted in the vicinity of lesions, and often they are severely distorted. Diseased fleshy roots whether misshapen or not are unmarketable. Lesions developing on the subterranean stems and fibrous roots cause a reduction in plant vigor and loss in stand especially under conditions of drought.

Soil rot is most severe in soils with a reaction near neutrality and becomes progressively less severe as soil acidity is increased. Unfortunately, detailed accounts of soil rot incidence in fields treated with sulfur have not been published. Yield data following sulfur treatment are more readily obtained. As early as 1900, Halsted (1) reported that sulfur was an effective material for soil treatment in New Jersey. His report was based upon trials conducted over a 4-year period beginning in 1895. In the same state, Poole (8) presented additional data showing that sulfur as a soil treatment was effective in reducing soil rot and scurf (*Monilochaetes infuscans* Ell. and Hals.). Yields were not impaired with 600 lbs. of sulfur per acre.

Manns and Adams (5) summarized the observations from Delaware. Broadcast applications of sulfur gave practical disease control, whereas soil reactions above pH 6.0 were conducive to soil pox. Sulfur, up to 400 lbs. per acre, reduced incidence of soil rot and scurf (4). Yields of plots receiving 400 lbs. of sulfur per acre in 1922 were above those of the untreated plots.

In Indiana (2) soil rot was reduced by the use of sulfur and increased by use of lime on sandy soils having an initial reaction around pH 6.3 to 6.9. Increasing the acidity with sulfur to about pH 5.9 or 6.0 greatly reduced soil rot and decreased yield little or not at all. Soil rot was prevalent and yields were relatively low at pH 7.5 and above.

The most detailed report of extensive field trials with sulfur as a soil treatment for soil rot control is that presented by Person (6) in Louisiana. Rates of 500 to 800 lbs. per acre were most effective for increased yields and soil rot control. In wet seasons there was little increase in yields of plots which were sulfured whereas in summers of average rainfall yield increases were marked. In dry seasons yields in untreated plots were extremely low while satisfactory yields were obtained following sulfur treatment. At levels below pH 5.1 soil rot was not important.

This paper is a report of trials with sulfur in Iowa sweet potato fields known to be heavily infested with the soil rot organism and in other fields known to be free of it. Quantitative data were obtained in order to make comparisons between soil rot incidence and total yield and to determine the relationship existing between soil pH and disease severity.

MATERIALS AND METHODS.—Finely ground elemental sulfur was broadcast to replicated plots in which the smallest unit was at least 20 × 46 ft. Wherever possible a Latin square plot design was used, although in certain cases, a randomized block design was necessary.

In early spring, sulfur was broadcast by hand and later thoroughly disked into the soil. Growers followed their own fertilizer practice, usually 500 lbs. of 3-9-18 or 3-12-12 per acre. Sulfur rates were none, 400, 800, and 1200 lbs. per acre except at the Southeastern Iowa Experiment Farm, Conesville, Iowa, where the highest rate was 1600 lbs. In 1947, dusting sulfur 325 mesh was used whereas in 1948 and 1949, ground sulfur, 80 per cent of which was 65 mesh or smaller, was applied.

The hydrogen-ion concentration of the soil was determined from a composite soil sample made by mixing 4 or 5 smaller samples taken at random from each plot at depths of from 2 to 4 in. These were suspended in enough water to make a semi-fluid suspension. Measurements were made electrometrically with a glass electrode after the hydrogen-ion concentration had become stable. The reaction listed in the table thus indicates the pH of the soil in which the fleshy roots developed rather than the reaction of the soil in which the entire root system was located.

Growers planted slips of the varieties of Little Stem Jersey types which they had used for other plantings on their farms.

Disease indices were calculated after roots were rated as: (0) healthy—those showing no disease; (1) slight—those with only 1 small lesion; (2) moderate—

[1] Accepted for publication July 11, 1952.

Journal Paper Number J-2033 of the Iowa Agricultural Experiment Station, Ames, Iowa, Project 1103, supported in part by a grant in aid from the Texas Gulf Sulphur Company, Houston, Texas. The authors are indebted to Harold Bartenhagen, Harold Butcher, J. L. Greiner, George Shoultz, and W. W. Singleton for excellent cooperation in maintaining field plots on their own farms. The advice and counsel of J. G. Darroch formerly of the Statistical Laboratory, Iowa Experiment Station is gratefully acknowledged. Photographs were prepared by John Staby.

[2] Associate Professor of the Department of Botany and Plant Pathology and Associate of the Department of Horticulture respectively.

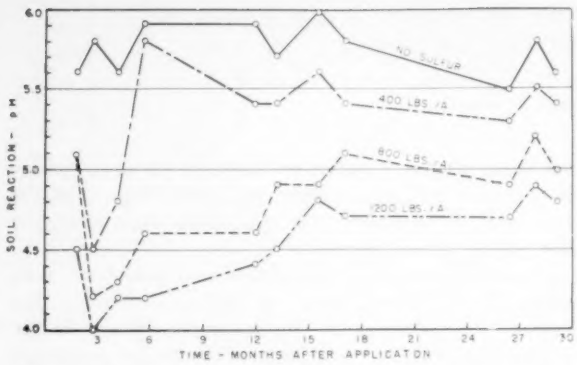



FIG. 1. Changes in soil reaction after broadcasting sulfur on Buckner coarse sand at the Muscatine Island Field Station, Fruitland, Iowa in April 1947.

those with more than 2 lesions but not severely affected; and (3) severe—those with 5 or more well established lesions or with lesions causing irregular root shape (Fig. 2). The number of roots in each group was multiplied by the rating of the group and the products added. Indices were expressed as percentages after dividing the sum by the product of the total number of roots and, 3, the number of the highest disease category. Reduced to a formula this is as follows:

$$\text{Disease Index} = \frac{100\,[\,(\text{no. healthy} \times 0) + (\text{no. slight} \times 1) + (\text{no. moderate} \times 2) + (\text{no. severe} \times 3)\,]}{3\ (\text{total no. of roots examined})}$$

Roots were graded into U. S. #1 and #2 sizes, the former being at least 1.75" in diameter and the latter between 1.5 and 1.75" in diameter. Total yields were calculated without any reduction due to root disfigurement by soil rot. Marketable yields represent the yield of #1 size roots placed in the healthy and slightly diseased groups, whereas roots placed in the moderately and severely diseased categories were considered culls.

Yield data were treated statistically using analysis of variance. A covariance study of soil pH and soil rot indices was made after the method described by Snedecor (9).

EXPERIMENTAL RESULTS.—Sulfur was broadcast to plots on Buckner coarse sand at the Muscatine Island Field Station in April 1947. Disease records, yield, and soil pH data were taken during the following 3 growing seasons. Soil pH levels (Fig. 1) dropped rapidly following sulfur treatment and within 3 months were pH 4.5 or lower whereas untreated plots were above pH 5.5. Soil pH levels remained low in the 800 and 1200 lb. sulfur plots throughout the season. In subsequent years, soil pH levels shifted gradually toward neutrality but remained distinctly below the untreated plots throughout the 3-year period of observation. In contrast, soil in the plots receiving 400 lbs. of sulfur rapidly shifted toward neutrality and by the end of the season was within 0.2 pH units of the untreated control.

Total yields in 1947 (Table 1) were very low on the untreated plots because of cold weather in the early part of the season and drought during the summer. Yields from the 800 and 1200 lb. treatments were significantly lower than from the untreated plots indicating that pH levels below 4.5 following sulfur treatment had been unfavorable for sweet potato growth at this location. There were no detectable differences in vigor of vine growth throughout the season.

The area on which the plots were located was known to be heavily infested with soil rot. In this particular season, soil rot incidence on the fleshy roots was so low that disease indices could not be calculated. Since soil rot was severe in subsequent years it seems probable that inoculum was present but that environmental conditions during the season had been unfavorable for infection.

In 1948, conditions were rather favorable but not optimum for sweet potato growth. In the untreated plots, soil rot on fleshy roots was severe (Fig. 2) and total yields of both varieties were relatively satisfactory. Soil pH levels were reduced by each increment of sulfur. There was a general trend with both varieties particularly with the 800 and 1200 lb. rates for total yield increases following sulfur treatment. Vines were slightly more vigorous with 800 and 1200 lbs. of sulfur than where lower rates were used. Disease indices were reduced and the percentage of marketable crop increased with each increment of sulfur. Disease was not reduced to a satisfactory level with 400 lbs. of sulfur, but was well controlled at the 800 lb. level; (Fig. 3) there was virtually no soil rot at the 1200 lb. rate. Disease indices of the 2 varieties tested were virtually the same. Yields of marketable #1 size roots were significantly increased in the 800 and 1200 lb. plots.

The third season after broadcasting sulfur, soil pH levels remained between 4.5 and 5.0 with 1200 lbs. of sulfur. Disease incidence was generally lower than in the previous season. Soil rot was progressively reduced as sulfur rates were increased. Vines in the 1200 lb. plots were slightly more vigorous than those with no sulfur or lower rates of sulfur. Total yields were generally increased as sulfur rates were increased, being significantly greater than the untreated controls with 1200 lbs. of sulfur. Marketable yields from the 800 and 1200 lb. rates were significantly higher than those from the 400 lb. sulfur rates or from the untreated plots.

Sulfur was broadcast in 1948 on the Harold Butcher farm 3 miles northeast of the Muscatine Island Field Station in a soil of essentially similar type but containing more gravel. Soil rot had been so severe a few years previously that the field had been abandoned for sweet potato culture. Yields were low because the experimental area, which was in an isolated portion of the farm, was not irrigated through the season. At harvest, the soil reaction was below pH 4.5 in all sulfur plots and with the 1200 lb. rate, pH levels approaching 3.5 were obtained. There were no consistent dif-

TABLE 1.—*Influence of broadcast sulfur on sweet potato yields and soil rot incidence in the coarse sandy soils near Muscatine, Iowa*

| Year of observation and variety | Rate of sulfur | Soil reaction [a] early season | Soil reaction [a] at harvest | Soil rot index | Total yield [b] #1 size roots | Total yield [b] #1 and #2 size roots | Marketable yield of #1 size roots | |
|---|---|---|---|---|---|---|---|---|
| | *lbs./A.* | *pH* | *pH* | *Percent* | *bu./A.* | *bu./A.* | *Percent* | *bu./A.* [c] |
| Muscatine Island Field Station, Fruitland, Iowa, Sulfur broadcast April 1947 | | | | | | | | |
| 1947 | none | 5.6±0.3 | 5.9±0.2 | † | 11.2 | 34.4 | ----- | ----- |
| Shoemaker | 400 | 5.1±0.4 | 5.8±0.3 | † | 10.0 | 28.6 | ----- | ----- |
| | 800 | 5.1±0.1 | 4.6±0.4 | † | 9.5 | 23.2 * | ----- | ----- |
| | 1200 | 4.5±0.6 | 4.2±0.0 | † | 7.9 | 19.9 * | ----- | ----- |
| | | | | LSD [d] 5% | | 10.2 | | |
| | | | | LSD 1% | | 15.5 | | |
| 1948 | none | 5.7±0.1 | 5.8±0.2 | 73 | 32.7 | 55.0 | 15.3 | 5.0 |
| Muscatine | 400 | 5.4±0.1 | 5.4±0.2 | 57 | 40.2 | 63.7 | 40.3 | 16.2 |
| Jersey | 800 | 4.9±0.3 | 5.1±0.2 | 22 | 48.5 | 76.5 | 83.3 | 40.4 * |
| | 1200 | 4.5±0.3 | 4.7±0.1 | 11 | 46.0 | 73.2 | 90.4 | 41.6 ** |
| | | | | LSD 5% | | 29.0 | | 23.6 |
| | | | | LSD 1% | | 43.9 | | 35.8 |
| 1948 | none | 5.7±0.1 | 5.8±0.2 | 75 | 30.2 | 49.0 | 18.2 | 5.5 |
| Orange | 400 | 5.4±0.1 | 5.4±0.2 | 60 | 37.2 | 56.7 | 28.5 | 10.6 |
| Little | 860 | 4.9±0.3 | 5.1±0.2 | 19 | 53.0 | 81.0 * | 82.8 | 43.9 ** |
| Stem | 1200 | 4.5±0.3 | 4.7±0.1 | 5 | 49.2 | 72.0 | 93.5 | 46.0 ** |
| | | | | LSD 5% | | 27.6 | | 21.0 |
| | | | | LSD 1% | | 41.8 | | 31.8 |
| 1949 | none | 5.5±0.1 | 5.6±0.4 | 53 | 70.5 | 117.7 | 47.5 | 33.5 |
| Orange | 400 | 5.3±0.2 | 5.4±0.2 | 30 | 79.2 | 135.2 | 76.3 | 60.4 |
| Little | 800 | 4.9±0.2 | 5.0±0.2 | 8 | 84.2 | 143.5 | 94.8 | 79.8 * |
| Stem | 1200 | 4.7±0.1 | 4.8±0.2 | 2 | 96.0 | 150.5 * | 100.0 | 96.0 ** |
| | | | | LSD 5% | | 28.0 | | 32.3 |
| | | | | LSD 1% | | 42.4 | | 48.9 |
| Harold Butcher Farm, Muscatine, Iowa, Sulfur broadcast April 1948 | | | | | | | | |
| 1948 | none | 5.6±0.1 | 5.7±0.2 | 9 | 7.5 | 20.7 | 90.7 | 6.8 |
| Orange | 400 | 5.4±0.5 | 4.2±0.4 | 5 | 20.0 | 40.2 | 93.0 | 18.6 * |
| Little | 800 | 5.5±0.3 | 4.0±0.6 | 6 | 27.5 | 49.5 | 94.5 | 26.0 ** |
| Stem | 1200 | 5.1±0.4 | 3.5±0.2 | 2 | 24.7 | 42.5 | 100.0 | 24.7 ** |
| | | | | LSD 5% | | 29.1 | | 11.2 |
| | | | | LSD 1% | | 44.0 | | 16.9 |
| 1949 | none | 5.7±0.1 | 5.6±0.2 | slight | 43.5 | 70.7 | ----- | ----- |
| Orange | 400 | 5.4±0.4 | 4.8±0.4 | trace | 52.5 | 81.2 | ----- | ----- |
| Little | 800 | 4.8±0.1 | 4.3±0.7 | none | 58.0 | 83.0 * | ----- | ----- |
| Stem | 1200 | 3.9±0.6 | 3.9±0.6 | none | 31.0 | 40.5 ** | ----- | ----- |
| | | | | LSD 5% | | 11.7 | | |
| | | | | LSD 1% | | 17.7 | | |
| George Shoultz Farm, Muscatine, Iowa, Sulfur broadcast April 1948 | | | | | | | | |
| 1948 | none | 6.0±0.4 | 6.3±1.2 | 41 | 73.0 | 152.5 | 56.0 | 40.9 |
| Menentico | 400 | 5.1±0.0 | 4.7±0.4 | 8 | 88.7 | 151.7 | 92.1 | 81.7 ** |
| Jersey | 800 | 4.8±0.2 | 4.0±0.6 | 6 | 85.7 | 148.7 | 95.0 | 81.4 ** |
| | 1200 | 4.5±0.2 | 3.6±0.1 | 6 | 82.0 | 116.2 ** | 93.8 | 76.9 ** |
| | | | | LSD 5% | | 25.1 | | 15.1 |
| | | | | LSD 1% | | 36.1 | | 22.8 |

[a] Soil reaction at the 2 to 4" depth. The first figure indicates the mean from the 4 plots and the second figure indicates the greatest variation from the mean.

[b] U. S. #1 size roots were those over 1.75" in diameter, whereas #2 size roots were between 1.5 and 1.75" in diameter.

[c] Marketable yields are the products of individual plot yields of #1 size roots and the percentage of crop which was marketable.

[d] Least difference required for significance at the 5 and 1 per cent levels.

† Insufficient soil rot for calculation of disease index.

* Yields significantly different from untreated control at the 5 per cent level; ** at the 1 per cent level.

ferences in vine growth during the season. Soil rot incidence was very low in the entire plot although disease was slightly more severe in the untreated soil than in the sulfured plots. The first season there was a consistent increase in total yield from sulfur treated plots. Marketable yields were significantly increased with each level of sulfur applied.

In the second season, since there was virtually no

FIG. 2. Representative roots of the Muscatine Jersey variety grown on the Muscatine Island Field Station from plots receiving no sulfur. Roots were arranged in order of increasing severity starting in the upper left hand corner and reading from left to right in each row. The first 2 were rated healthy (group 0), the next 2 slight (group 1), the next 5 moderate (group 2), and the last 11 severe (group 3).

soil rot on the fleshy roots, marketable yields were equal to total yields. Total yields at the 800 lb. rate were significantly increased over the untreated plots while at the 1200 lb. level there was a significant yield reduction. Plant vigor was generally low in the 1200 lb. sulfur plots and slightly greater in the 400 and 800 lb. levels than in the untreated plots. Soil pH levels with 1200 lbs. of sulfur were approximately 3.9 through the season and growth was poor. This is in contrast to results of the previous season in which pH levels were below 4.0 in July and September and yields were unimpaired.

Confirmatory data were obtained in 1948 on soil of similar type at the George Shoultz farm 2 miles northeast of the Butcher plots. Soil rot was severe on the untreated plots and slight where sulfur had been applied. Total yields from the 400 and 800 lb. rates were similar to those from the untreated plots. Soil pH levels following 1200 lbs. of sulfur were below 3.8 in July, August and September and yields were significantly reduced. In contrast, marketable yields with all rates of sulfur were significantly higher than from the untreated plots. Thus, sweet potato yields with 1200 lbs. of sulfur had been reduced but the loss in yield had been compensated by increased quality attributable to soil rot control.

On some farms, soil rot was known to be severe in fields which had been heavily limed or treated with ground clam shells from the local pearl button factory. To investigate this phase of the problem, sulfur plots were prepared on the farms of J. L. Greiner and Harold Bartenhagen. Sulfur rates up to 1200 lbs. per acre were not effective in altering soil pH levels appreciably and disease control was not obtained. Higher rates of sulfur were not tested because of the danger of making the sandy soil too acid in areas where the lime may have been unevenly applied.

In the Conesville area, approximately 25 miles southeast of Muscatine, soil rot has recently become a problem. This soil is a Buckner loamy sand on which irrigation is not usually practiced. In a field where soil pox was rather uniformly distributed, sulfur plots were

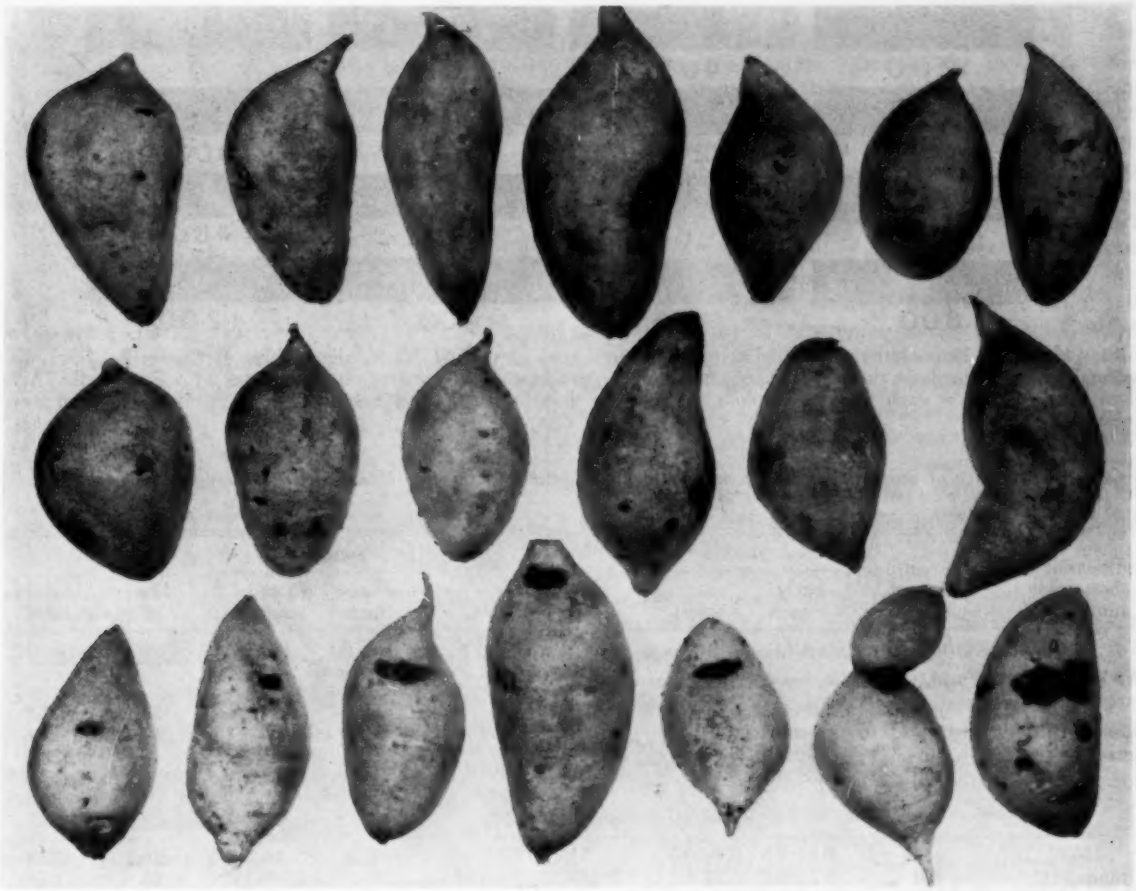


FIG. 3. Representative roots of the Muscatine Jersey variety grown on the Muscatine Island Field Station from plots receiving 800 lbs. of sulfur broad cast in the spring of the previous growing season.

prepared in 1948 and replanted without further treatment in 1949. The soil reaction (Table 2) was originally very near neutrality and was changed to approximately pH 5 with the high rates of sulfur. Disease indices of the varieties Menentico and Orange Little Stem were reduced with each increment of sulfur and total yields as well as marketable yields were consistently increased following treatment with 800 and 1200 lbs. per acre. Early in the 1949 growing season rainfall was low and temperatures rather high. Stand losses which reflect the severity of the soil rot disease on the underground stems and fibrous roots of the sweet potato plants were severe in the untreated plots and in those receiving 400 lbs. of sulfur whereas vegetative growth was much more vigorous at the 800 and 1200 lb. levels (Fig. 4).

Sulfur plots were prepared on the Southeastern Iowa Experiment Association farm located on a similar soil type ½ mile north of the Singleton farm. Since soil rot was known to be absent from this field, sulfur was broadcast to determine its effect on sweet potatoes in the absence of disease. Rates of none, 400, 800, and 1600 lbs. of sulfur were tested. The reaction of untreated soil was rather acid being between pH 5.0 and 5.5. At the close of the season, pH levels below 4.0 were obtained following 800 and 1600 lbs. of sulfur. At these pH levels, yields were significantly lower than those of the controls and at the 1600 lb. level plants were definitely stunted. Sweet potatoes were planted the following season without further additions of sulfur and similar results were obtained. Sweet potato injury was more severe with 800 and 1600 lbs. of sulfur the second season than it was the first season after application although soil pH levels at harvest were relatively the same.

Soil rot indices were plotted against soil reaction at time of harvest from data obtained in 1948 and 1949 on 2 different sandy soils, 1 at Conesville, and 1 at Muscatine in southeastern Iowa. Treatment means and regression equations are in Figure 5. A covariance analysis, according to the method described by Snedecor (9) (Table 3) was carried out for both years at the Muscatine Island Field Station and at the W. W. Singleton farm. Analyses were not made on data from other locations because of low disease indices. There were no significant differences between the slopes of regression lines obtained at the Muscatine Island Field Station in 2 different years. However

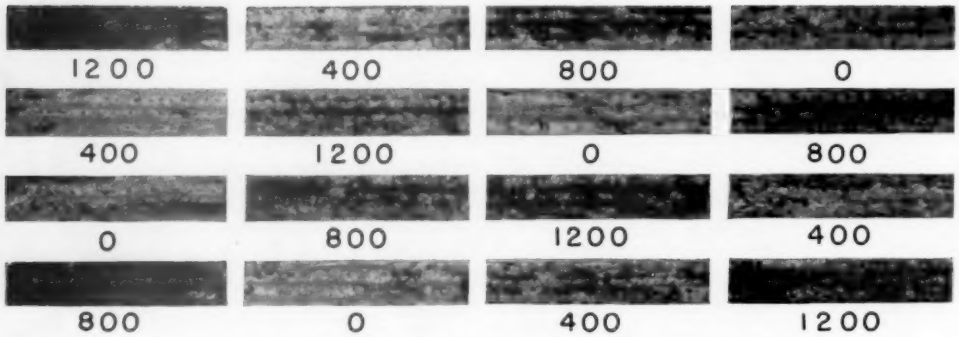



Fig. 4. Aerial view of the soil rot plots on the loamy sand of the W. W. Singleton farm at Conesville, Iowa where sulfur had been broadcast the previous season. Plot borders have been removed and the rates of sulfur in lbs./A. are shown directly below each plot. Dark colored plots are those in which vines are vigorous whereas exposed sand is light colored.

TABLE 2.—*Influence of broadcast sulfur on sweet potato yields and soil rot incidence in the loamy sand-soil at Conesville, Iowa*

| Year of observation and variety | Rate of sulfur | Soil reaction [a] early season | Soil reaction [a] at harvest | Soil rot index | Total yield [b] #1 size roots | Total yield [b] #1 and #2 size roots | Marketable yield of #1 size roots | |
|---|---|---|---|---|---|---|---|---|
| | *lbs./A.* | *pH* | *pH* | *Percent* | *bu./A.* | *bu./A.* | *Percent* | *bu./A.*[c] |
| W. W. Singleton Farm, Conesville, Iowa, Sulfur broadcast April 1948 | | | | | | | | |
| 1948 | none | 6.8±0.3 | 6.9±0.3 | 68 | 66.0 | 102.2 | 26.1 | 17.2 |
| Menentico | 400 | 6.5±0.2 | 6.2±0.7 | 57 | 78.2 | 118.5 | 37.8 | 29.6 |
| Jersey | 800 | 6.0±0.4 | 5.2±0.7 | 47 | 110.7 | 162.2 * | 48.1 | 53.2 |
| | 1200 | 6.3±0.3 | 5.1±1.3 | 44 | 116.2 | 162.0 * | 52.1 | 60.6 * |
| | | | | LSD 5%[d] | | 49.7 | | 36.4 |
| | | | | LSD 1% | | 75.3 | | 55.1 |
| 1948 | none | 6.8±0.3 | 6.9±0.3 | 51 | 48.5 | 86.2 | 46.2 | 22.4 |
| Orange | 400 | 6.5±0.2 | 6.2±0.7 | 34 | 95.7 | 141.2 | 68.5 | 65.6 |
| Little | 800 | 6.0±0.4 | 5.2±0.7 | 35 | 70.0 | 121.0 | 63.0 | 44.1 |
| Stem | 1200 | 6.3±0.3 | 5.1±1.3 | 23 | 93.2 | 142.7 | 79.3 | 73.9 * |
| | | | | LSD 5% | | 73.7 | | 45.9 |
| | | | | LSD 1% | | 111.6 | | 69.5 |
| 1949 | none | 6.0±0.5 | 6.4±0.5 | 65 | 14.7 | 18.5 | 27.9 | 4.1 |
| Orange | 400 | 5.9±1.1 | 6.2±0.5 | 60 | 12.5 | 14.5 | 37.6 | 4.7 |
| Little | 800 | 5.8±0.4 | 5.4±0.9 | 47 | 61.5 | 80.0 ** | 47.1 | 29.0 * |
| Stem | 1200 | 5.4±1.5 | 4.8±0.7 | 29 | 54.0 | 77.0 ** | 70.7 | 38.2 ** |
| | | | | LSD 5% | | 28.7 | | 18.1 |
| | | | | LSD 1% | | 43.4 | | 27.3 |
| Southeastern Iowa Experiment Association, Conesville, Iowa, Sulfur broadcast April 1948 | | | | | | | | |
| 1948 | none | 5.3±0.1 | 5.1±0.1 | none | ..... | 71.7 | ..... | ..... |
| Nancy Hall | 400 | 4.9±0.5 | 4.0±0.3 | none | ..... | 73.3 | ..... | ..... |
| | 800 | 4.7±0.3 | 3.9±0.3 | none | ..... | 37.3 * | ..... | ..... |
| | 1600 | 4.6±0.1 | 3.4±0.1 | none | ..... | 31.7 * | ..... | ..... |
| | | | | LSD 5% | | 33.5 | | |
| | | | | LSD 1% | | 50.7 | | |
| 1949 | none | 5.0±0.2 | 5.0±0.1 | none | ..... | 97.0 | ..... | ..... |
| Orlis | 400 | 4.8±0.4 | 4.3±0.2 | none | ..... | 104.0 | ..... | ..... |
| | 800 | 4.0±0.2 | 3.8±0.1 | none | ..... | 34.0 * | ..... | ..... |
| | 1600 | 3.5±0.4 | 3.6±0.1 | none | ..... | 5.7 ** | ..... | ..... |
| | | | | LSD 5% | | 54.9 | | |
| | | | | LSD 1% | | 83.1 | | |

[a] Soil reaction at the 2 to 4" depth. The first figure indicates the mean from the 4 plots and the second figure indicates the greatest variation from the mean.

[b] U. S. #1 size roots were those over 1.75" in diameter, whereas #2 size roots were between 1.5 and 1.75" in diameter.

[c] Marketable yields are the products of individual plot yields of #1 size roots and the percentage of crop which was marketable.

[d] Least difference required for significance at the 5 and 1 per cent level.

* Yields significantly different from untreated control at the 5 per cent level; ** at the 1 per cent level.

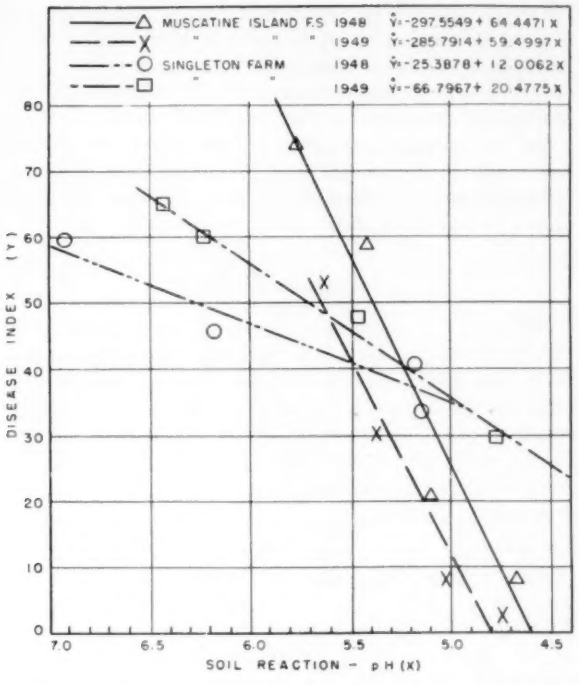



FIG. 5. Soil rot incidence plotted against soil reaction at time of harvest on two sandy soil types in southeastern Iowa. Regression equations were calculated from treatment means.

there were significant differences between the regression lines obtained on the Singleton farm in the 2 different seasons. Differences in slopes of regression lines between the 2 locations were highly significant.

Records of stem rot incidence (*Fusarium oxysporum* f. *batatas* (Wr.) S. and H.) were made in 1948 and 1949 at the farm of the Southeastern Iowa Experiment Association and at the W. W. Singleton farm. At no time was there any evidence that the amount of stem rot was either reduced or increased by sulfur treatment.

No data were obtained concerning the influence of either sulfur or soil acidity on black rot (*Ceratostomella fimbriata* (E. and H.) Ell.) and scurf (*Monilochaetes infuscans* Hals.) since neither disease was present.

Observations were made on flavor of potatoes grown in sulfur treated soil. At no time could any difference in flavor be detected between potatoes from sulfured plots and from the untreated controls.

DISCUSSION.—A regression study was made of soil rot incidence and soil pH levels at 2 locations and significant differences were obtained between the slopes of the regressions at these locations. It is possible that differences in the soil type may have been partially associated with these differences in regressions. The steepest regression was obtained from plots at the Muscatine Island Field Station which were situated on a coarse sand extremely low in organic matter. Regressions were more gradual at the Singleton farm 30 miles distant, the soil of which is a loamy sand, low in organic matter but higher than at the first mentioned location. Significant differences in yearly regressions at the W. W. Singleton farm suggest that seasonal differences were also important. The possibility exists that the cumulative effect of soil acidity may have been more detrimental to the soil rot organism the second year after application than the first. Further comparison with results from other states is impossible because of lack of quantitative disease data.

Sulfur has been commonly used to control potato scab incited by *Streptomyces scabies* (Thaxt.) Waksman and Henrici, a similar disease of the Irish potato. A regression study of scab incidence and soil pH was made on peat soils in northern Iowa at a number of locations (3). There were no significant differences in the slopes of various regressions at several locations. Regression slopes with potato scab were all considerably flatter than those obtained in this soil rot study.

Little is known concerning the nature of the action of sulfur on the soil rot organism. Presumably the acidification of the soil adversely affects *Streptomyces ipomoea* in the soil. In addition to the pH effect, it is possible that a toxic material may be produced in the soil having a direct effect upon the causal organism. Circumstantial evidence (10) suggests that the potato scab organism, *Streptomyces scabies*, is inhibited in sulfured peat soils by $H_2S$. This toxic effect of $H_2S$ is apparently distinct from that which later becomes effective as the soil acidity is increased.

The low disease indices from the sulfured plots on the Shoultz farm, regardless of the amount of sulfur applied and the high index obtained from the untreated plots, suggest a toxic effect somewhat different from the influence of hydrogen-ion concentration. The very steep regression slopes at the Muscatine Island Field Station suggest toxicity of sulfur or of sulfur derivatives which was somewhat distinct from the influence of hydrogen-ion concentration alone.

Person and Martin (7) obtained no growth of *Streptomyces ipomoea* in culture at pH 4.8 and only a trace of growth at pH 5.0. When the soil pH level in the

TABLE 3.—*Tests for homogeneity of regression between years within farms and between farms for both years*

| Location and years of test | Mean square | | F value |
|---|---|---|---|
| | Differences between years regression | Deviation from individual year regression | |
| Muscatine I. F. S. 1948 and 1949 | 25.9839 | 254.6276 | 0.1020 |
| W. W. Singleton farm 1948 and 1949 | 280.4961 | 48.4959 | 5.7839 * |
| Both locations both years | 7527.7972 | 151.8974 | 49.5584 ** |

* $P < 0.05$
** $P < 0.01$

field was lowered to 5.0 with an application of sulfur there was very little development of soil rot. They reported that the disease is ordinarily found in soils with the pH above 5.2. Person (6) later reported that the organism is not important at a soil pH of 5.0 or less. In Delaware, soil reactions (5) above pH 6.0 throughout the growing season were associated with increased soil rot and lower yields. In Indiana (2) soil pH levels below 5.9 or 6.0 greatly reduced soil rot and decreased yield little or not at all.

In Iowa, soil rot was present at soil pH levels somewhat below those reported elsewhere. The untreated plots at the Muscatine Island Field Station were below soil pH levels of 6.0 during the 3-year period of observation yet soil rot was as severe in this location as at any other in which sulfur tests were prepared. The highest average disease index obtained at any location was at the Muscatine Island Field Station in a soil of pH. 5.8. At soil pH levels between 5.5 and 5.0 in September, disease indices above 30 were obtained at this location and on the Singleton farm. Soil rot was observed at pH levels in September between 4.5 and 5.0 at the Muscatine Island Field Station, the Butcher farm, and the Shoultz farm.

Soil rot was known to be absent at the farm of the Southeastern Iowa Experiment Association and soil pH levels remained relatively stable throughout the second year after broadcasting sulfur. The critical pH levels in this soil above which satisfactory sweet potato yields were obtained and below which yields were unsatisfactory was somewhere between pH 4.3 and 3.8.

It was impossible to evaluate the influence of soil pH on sweet potato growth when soil rot was present since growth of the crop was influenced considerably by the degree of soil rot control.

Variation in soil rot incidence was considerable between seasons. Soil rot was known to be severe at the Muscatine Island Field Station yet the first year after plots had been prepared soil rot was virtually absent. In the 2 subsequent seasons, soil rot was severe in the same plots. A somewhat similar condition existed at the Butcher farm where soil rot was slight but present in 1948 and virtually absent in 1949. In the past, this area was known to be heavily infested. Variation of a similar type was observed with respect to the influence of soil rot on sweet potato stands. In only one location in one season, W. W. Singleton farm, 1949, were stands severely reduced by soil rot. Person (6) reported that stands were severely affected when drought occurred at the time young plants were producing roots. Little is known concerning the influence of seasonal variation in soil temperature and moisture on infection of fleshy roots by the soil rot organism.

Scurf (*Monilochaetes infuscans* Hals.) was absent in these plots and no information was obtained concerning the effect of sulfur soil treatment on the disease. Poole (8) and Manns and Adams (4) observed reduction in scurf following sulfur treatment.

Although stem rot (*Fusarium oxysporum* f. *batatas* (Wr.) S. and H.) counts were made throughout 2 seasons in the 2 plots at Conesville, there was no evidence that sulfur had influenced the incidence of stem rot. This is in agreement with the observation of Manns and Adams (5) who could find no influence of sulfur on stem rot but observed that black rot (*Ceratostomella fimbriata* (E. and H.) Ell.) was reduced following sulfur treatment. Black rot was virtually absent in the Iowa plots.

## SUMMARY

Soil rot is an important disease of sweet potatoes in the sandy soils of southeastern Iowa. Many fields have been abandoned for further sweet potato culture because of the disease. Reduced stands and plant vigor result from the attack of *Streptomyces ipomoea* on the fibrous roots and lower stems. Fleshy roots are disfigured by the lesions and often distorted.

Sulfur as a soil treatment was effective in controlling the disease. Total yields and plant vigor were often increased following sulfur treatment and the appearance of the fleshy roots was appreciably improved. Although disease was present in soils with pH as low as 4.7 practical control was usually obtained somewhere around pH 5.0. In these soils, total yields and plant vigor were adversely affected at approximately pH 4.0.

The 800 lb. sulfur rate provided conditions for optimum plant growth and satisfactory disease control. Maximum soil rot control was obtained with 1200 lbs. of sulfur, but in certain locations this rate was above the optimum for plant growth. Disease control was not satisfactory with 400 lbs. of sulfur.

There was no evidence that stem rot (*Fusarium oxysporum* f. *batatas*) had been influenced by variation in hydrogen-ion concentration of the soil.

Department of Botany and Plant Pathology
and
Department of Horticulture
Iowa Agricultural Experiment Station
Ames, Iowa

## LITERATURE CITED

1. Halsted, B. D. 1900. Soil fungicides for potato and turnip diseases. New Jersey Special Bul. 8.
2. Hartman, J. D., and R. W. Samson. 1940. Effect of soil reaction on soil rot of sweet potatoes. (Abs.) Phytopathology **30**: 8-9.
3. Hooker, W. J., and G. C. Kent. 1950. Sulfur and certain soil amendments for potato scab control in the peat soils of northern Iowa. Amer. Potato Jour. **27**: 343-365.
4. Manns, T. F., and J. F. Adams. 1924. Annual report of director, Department of plant pathology and soil bacteriology. Delaware Agr. Exp. Sta. Bul. **135**: 25-34.
5. Manns, T. F., and J. F. Adams. 1925. Annual report of director, Department of plant pathology. Delaware Agr. Exp. Sta. Bul. **141**: 24-26.
6. Person, L. H. 1946. The soil rot of sweet potatoes and its control with sulfur. Phytopathology **36**: 869-875.
7. Person, L. H., and W. J. Martin. 1940. Soil rot of

sweet potatoes in Louisiana. Phytopathology **30**: 913-926.
8. POOLE, R. FRANK. 1922. Recent investigations on the control of three important field diseases of sweet potatoes. New Jersey Agr. Exp. Sta. Bul. 365.
9. SNEDECOR, GEORGE W. 1946. Statistical methods applied to experiments in agriculture and biology. 4th edition. Iowa State College Press, Ames, Iowa.
10. VLITOS, A. J., AND W. J. HOOKER. 1951. The influence of sulfur on populations of *Streptomyces scabies* and other streptomycetes in peat soil. Amer. Jour. Bot. **38**: 678-683.

# STUDIES ON INTERNAL BROWNING OF TOMATO [1]

S. P. Raychaudhuri [2]

Haenseler (13) described a disease of tomatoes under the name of internal browning. This disease somewhat resembles vascular browning or gray wall reported by Conover (7) from Florida and internal browning or core rot reported by Young (29) from Texas. Gray wall of tomato was also reported by Lorenz and Knott (22) from California where it became a serious problem. During 1948 and 1949 Friedman (12) observed tomatoes with symptoms identical with those of internal browning disease on crops grown in Pennsylvania, New York, Florida, and New Jersey. Holmes (15, 16) presented evidence of the association of strains of tobacco-mosaic virus (*Marmor tabaci* H.) from plantain with internal browning of tomato occurring in New Jersey. Some of the literature has recently been reviewed by Huffington (17) who also discussed related ripening disorders of tomatoes.

Several features of internal browning disease have remained obscure. Affected tomato fruits are unfit for market, yet growers often wish to use certain selections of the same varieties for planting the crop in the next season. Consequently, the question has arisen as to whether it would be safe to use seeds from fruits collected in affected fields and whether strains of tobacco-mosaic virus found associated with internal browning would appear in seedlings subsequently grown from such seeds. The disease has not yet been reproduced under experimental conditions in mechanically inoculated plants (16). Could this mean that it might be dependent in part on the presence of some graft-transmissible, but not mechanically-transmissible, virus? The present investigation was concerned with 2 features of this problem: attempts to reproduce the disease experimentally, and tests on transmission of strains of tobacco-mosaic virus through tomato seed. Preliminary results of retention of certain strains of tobacco-mosaic virus in tomato seeds have been published (25).

MATERIALS AND METHODS.—Samples of diseased tomato fruits of the variety Rutgers, showing manifestations of internal browning, were first obtained from Dr. F. O. Holmes, who also kindly supplied the ordinary strain and the ribgrass strain of tobacco-mosaic virus (TMV) for comparison with isolates of this virus obtained from fruits affected by internal browning disease. Later, diseased fruits of the varieties Queens and Rutgers were collected from the College Farm of the New Jersey State Agricultural College at New Brunswick and from certain other plots in this neighborhood, as well as from Bridgeton, New Jersey. Diseased tomatoes of the variety Jubilee were kindly supplied by Dr. Edwin E. Honey of Pennsylvania State College, State College, Pennsylvania, Mr. Milton Cowan of Middlesex County, New Jersey, and the W. Atlee Burpee Company, Doylestown, Pennsylvania.

Seeds collected from all tomato varieties were exposed to fermentation at room temperature for 3 days, washed thoroughly in running water and dried for further experimental work. Seeds of the Bonny Best variety of tomato purchased from commercial seed houses were used for raising seedlings in the greenhouse for grafting experiments. Seeds of the Rutgers, Valiant, Bonny Best, and Jubilee varieties of tomato purchased from commercial seed houses were used in testing the inhibitory effect of extracts of germinating tomato seeds on TMV.

*Nicotiana glutinosa* L. was used as the test plant in all cases to detect the possible presence of TMV in seedlings raised from diseased fruits.

ATTEMPTS TO REPRODUCE INTERNAL BROWNING IN TOMATO FRUITS BY GRAFTING.—In all grafting experiments scions were taken from Queens and Rutgers tomato plants bearing fruits with typical internal browning. Virus-free Bonny Best tomato seedlings about 23 to 30 cm. in height, raised in the greenhouse at 20° to 25° C., were used as healthy stocks. Cleft-grafting was employed in all cases.

Altogether, 32 grafts were made during the period August, 1950 to May, 1951. The grafted plants, which were under observation for 4 to 6 months, developed mottled leaves on new growth from the stock; in this respect they resembled diseased tomato plants in the field. However, the internal browning symptom did not develop. This indicates that some as yet unknown factor or factors are required for production of typical disease symptoms.

SEROLOGICAL REACTIONS.— Tests[3] were carried out

[1] Accepted for publication July 7, 1952.

[2] Visiting Investigator, the Laboratories of the Rockefeller Institute for Medical Research, New York, on study leave from the Indian Agricultural Research Institute, New Delhi, India.

The author is much indebted to Drs. L. O. Kunkel, F. O. Holmes, and L. M. Black for helpful criticisms. The cooperation of Dr. Edgar G. Rex, Plant Pathologist, New Jersey State Department of Agriculture, Trenton, New Jersey, has been very much appreciated.

[3] Thanks are due to Dr. Helen Purdy Beale for her kind cooperation during this experiment.

to determine whether or not the virus, apparently a strain of TMV, associated with internal browning is serologically related to TMV. Extracts of infective sap, at a dilution of 1:10, from leaves of diseased tomato plants that were inoculated with the strain of TMV associated with internal browning, were centrifuged at 3500 r.p.m. for 15 minutes. The supernatant fluid was tested with TMV antiserum (1:10) from the horse, employing the method described by Purdy (24). Heavy flocculent precipitates of varying amounts settled out in all the tubes containing the antiserum and the extracts, while no such precipitates were formed in the tubes containing the antiserum without the extract, nor in tubes containing only the extract and no antiserum. The serum reaction, therefore, showed that the infective juice of diseased plants contained TMV.

RETENTION OF TOBACCO-MOSAIC VIRUS IN TOMATO SEED.—Diseased tomatoes from which seeds were collected were first tested for presence of TMV by inoculating leaves of healthy *Nicotiana glutinosa* plants. The test plants produced characteristic necrotic local lesions within 3 to 4 days after inoculation, indicating the presence of TMV in the diseased tomatoes.

Electron micrographs[4] of extracts of diseased tomatoes were studied. Typical rod-shaped particles characteristic of TMV were observed. This finding bears out those of Knight and Oster (20) who found such particles in all strains of TMV.

Seeds from diseased tomatoes, after exposure to fermentation and drying, were germinated in the greenhouse at weekly or bi-weekly intervals until the sixteenth week. Extracts from 10 batches each of 10 seedlings raised from such seeds were tested on 3 vigorously growing healthy leaves of a *Nicotiana glutinosa* plant. Only seedlings raised from seeds stored for 1 week were found infected; those raised from seeds stored for periods of 2, 3, 4, 5, 6, 8, 10, 12, 14, and 16 weeks were healthy. In contrast, seedlings raised from seeds of Bonny Best tomatoes affected by the ordinary strain of TMV and stored for the same periods were found to be infected when tested after 1, 2, and 3 weeks and after 27 days, while those raised from seeds stored for the longer periods were healthy.

The tests described above were repeated 4 times with the seeds collected from tomatoes affected by internal browning and once with the ordinary strain of TMV, employing the same plan of experimentation with regard to the germination of seeds at intervals of 1 or 2 weeks. Essentially consistent results were obtained in all these tests.

At weekly intervals for 5 weeks, a batch of 100 dry seeds from tomatoes affected by internal browning was washed with 2 cc. of distilled water and this water was tested for presence of TMV by inoculating *Nicotiana glutinosa* plants. The dry seeds retained the virus externally for 2 weeks but not for 3, 4, or 5 weeks. Moreover, only 27 local lesions developed on 30 leaves of 10 *N. glutinosa* plants inoculated with the washings from seed stored for 2 weeks, while 244 and 92 local lesions, respectively, developed on similar sets of test plants inoculated with the washings from seed stored for 0 and 1 week. Hence, the amount of virus was very much reduced, although retained externally for 2 weeks.

COMPARISON OF A STRAIN OF TMV ASSOCIATED WITH INTERNAL BROWNING OF TOMATO WITH THE ORDINARY AND RIBGRASS STRAINS OF THIS VIRUS.—On 2 occasions sets of 8 plants each of *Nicotiana tabacum* L., *N. sylvestris* Speg., and *N. glutinosa* L. were inoculated with the ordinary strain of TMV, the ribgrass strain, and a strain associated with internal browning, respectively, using 320-mesh carborundum as an abrasive.

The virus present in tomato fruits that showed internal browning produced dark brown necrotic local lesions but no mottling in *Nicotiana sylvestris* (Fig. 1,C). In this respect it differed from the ordinary strain of TMV. It produced mosaic mottling in *N. tabacum* but not necrotic ring primary lesions (Fig. 1,A), thus differing from the ribgrass strain (14). Symptoms produced by this strain in *N. glutinosa* are in Figure 1,B.

INHIBITORY EFFECT OF EXTRACTS OF GERMINATING SEEDS OF TOMATO VARIETIES ON TMV.—Experiments were made with extracts of healthy germinating seeds of tomato varieties Rutgers, Valiant, Bonny Best, and Jubilee in an effort to discover why the virus disappeared from stored seeds. The infective plant juice and the extracts of germinating tomato seeds were clarified by centrifuging at 3,500 r.p.m. for 15 minutes. Mixtures in the ratios 1:4, 1:20, and 1:50 of infective plant juice and extracts of germinating seeds were inoculated onto half-leaves of *Nicotiana glutinosa*. In each experiment a set of equal numbers of half-leaves was inoculated with the infective sap diluted comparably with distilled water instead of with extracts of germinating tomato seeds. All test plants within an experiment were of approximately the same age and size. Results are in Table 1.

TABLE 1.—*Inhibitory effect of extracts of germinating tomato seeds on tobacco-mosaic virus*

| Tomato variety | Experiment I | | Experiment II | |
|---|---|---|---|---|
| | Ratio of infective juice to seed extract | Lesions in per cent of control | Ratio of infective juice to seed extract | Lesions in per cent of control |
| Rutgers | 1:4 | 35.3 | 1:20 | 26.5 |
| | 1:20 | 30.6 | 1:50 | 7.4 |
| Valiant | 1:4 | 41.2 | 1:20 | 27.4 |
| | 1:20 | 29.6 | 1:50 | 7.0 |
| Bonny Best | 1:4 | 43.6 | 1:20 | 24.0 |
| | 1:20 | 26.0 | 1:50 | 9.0 |
| Jubilee | 1:4 | 82.0 | 1:20 | 84.0 |
| | 1:20 | 64.0 | 1:50 | 53.0 |

[4] Electron micrographs were kindly prepared by Dr. Russell L. Steere.

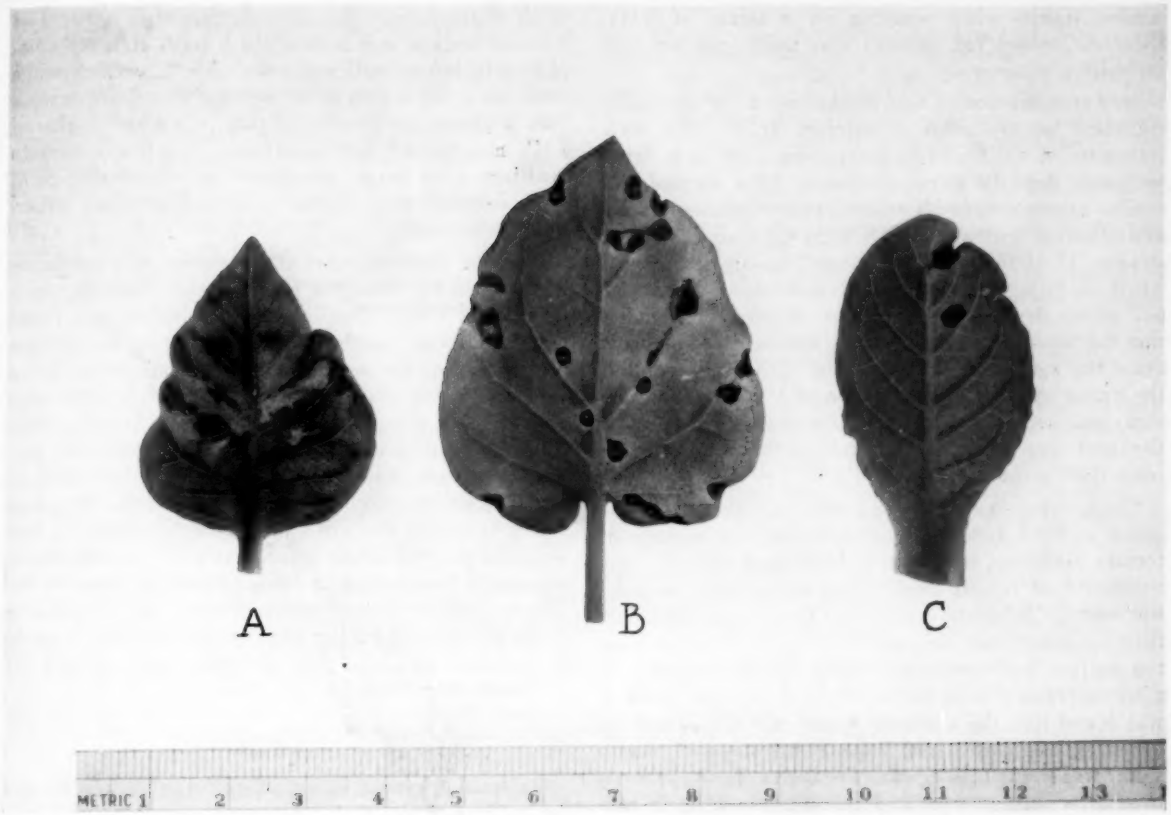



FIG. 1. Symptoms produced by a strain of tobacco-mosaic virus associated with internal browning of tomato: A) *Nicotiana tabacum*. B) *N. glutinosa*. C) *N. sylvestris*. (Photograph by J. A. Carlile.)

Extracts of varieties Rutgers, Valiant, and Bonny Best reduced the infectivity of the virus appreciably, while extracts of the variety Jubilee were not very effective in retarding the virus activity.

DISCUSSION.—The only report of the reproduction of internal browning under experimental conditions is that of Cox and Weaver (9). They grafted cuttings from diseased plants in the field to healthy tobacco and tomato plants in the greenhouse during August. Some fruits on the grafted tomato plants which ripened during November to December developed symptoms of internal browning, while all fruits ripening thereafter were symptomless. Stoner and Hogan (26) could not reproduce the disease occurring in Florida by grafting nor by mechanical inoculation and concluded that it is not caused by a transmissible pathogen. They suggested that some physiological factors might be responsible. Although Stoner and Hogan state in the title of their paper "graywall or internal browning of tomato," there may be a difference in the cause of the 2 diseases. During the course of investigations reported in the present article the disease could not be reproduced in tomato fruits. Further investigations are necessary in order to throw more light on the nature of this malady.

Chester (6) and Bawden and Pirie (2) distinguished certain strains of TMV serologically by employing precipitin absorption techniques, while Kleczkowski (19) differentiated strains by the amounts of virus required to reach equivalence with antiserum. Recently Weaver and Price (28) applied complement fixation reactions for distinguishing mutant strains of TMV. During the course of investigations reported in this paper, close serological relationship was established between a virus associated with internal browning and TMV. However, no attempt was made to differentiate the strain associated with internal browning from other strains of TMV by serological reactions.

Cook (8) and Bawden (1) reviewed comprehensive lists of the relevant literature on seed transmission of viruses. While many investigators raised healthy seedlings of tobacco and tomato from seeds collected from plants infected with TMV, others have reported transmission of the virus through the seed. Berkeley and Madden (3, 4) reported the transmission of mosaic through the seed of tomato. Doolittle and Beecher (10) obtained definite evidence of seed transmission of mosaic in tomato seeds planted soon after extraction from fruit. They observed the disease both in seedling and adult stages of the plant. However, after storage of the seeds for 3 to 12 months, they did not find disease symptoms in the seedling stage although 19 of 3,567 plants showed mosaic when 4 to 6 leaves developed on the plants. In a later publication they (11) reported

similar results while working on a strain of TMV (*Marmor tabaci* var. *siccans* var. nov.) causing leaf shrivelling of tomato.

Seed-transmission of this disease occurred in freshly extracted tomato seeds of infected fruits. No such transmission was found by them when seeds were dried for more than 10 days. Milbrath (23) carried out similar experiments with tomato variety Indiana Canner and observed that when seeds were sown after 3 weeks storage, 11 of 168 seedlings showed mosaic symptoms; when seeds were sown after 9 months storage, 5 of 677 plants developed the disease. Milbrath concluded that the tendency to transmit the mosaic virus depends upon the age of seeds. van Koot (21) observed that the extent of seed-borne infection by the tomato-mosaic virus was very slight (0.1 to 1.2 per cent) even when the seed was immersed for half an hour in infected juice the day before sowing.

Chamberlain and Fry (5) reported that the type strain of TMV is transmitted to a small percentage of tomato seedlings raised from uncleaned seeds. They succeeded in raising healthy seedlings after cleaning the seed by fermentation or HCl treatment. They further suggested that the seed carried the virus only on the surface and seedlings became infected during or after emergence from the seed. In the present work it was found that the ordinary strain of TMV as well as the strain associated with internal browning of tomato were transmitted to young seedlings through seeds even after fermentation and thorough washing in running water. However, the virus was not infective to seedlings in any of the tests after 5 weeks storage. Obviously, seeds collected from diseased fruits could be used for raising a healthy tomato crop if stored for this or longer periods. Presumably, longer storage would increase the margin of safety.

The decrease in seed transmission during storage of seeds from infected tomato plants is remarkable because some seed-transmitted viruses do not thus decrease although they have relatively low thermal inactivation points and are comparatively unstable when stored in extracts of infected plants. Thus, lettuce-mosaic virus is inactivated in 10 minutes at 55° to 60° C. and bean-mosaic virus has essentially the same degree of stability to heat. Neither of these viruses can be stored readily for long periods in dried tissues of infected leaves. It has not been shown, however, that infected lettuce seeds or bean seeds lose their respective viruses even on long storage. Valleau (27) observed that the yellow ring spot virus was retained in tobacco seed even after a period of 5½ years, although in this case a decrease in percentage of seed-transmission occurred during storage.

The present investigation indicates that the tendency of TMV to infect seedlings grown from seeds of diseased fruits may vary with the strain of virus present. Thus, ordinary TMV was found to infect seedlings grown from seeds stored for 3 and approximately 4 weeks in each of the 2 experiments, whereas the virus associated with internal browning of tomatoes failed in all of the 5 experiments to do this after either 3 or 4 weeks storage and in 4 of the 5 trials it failed completely to infect seedlings even after 2 weeks storage although in all 5 tests it did so after 1 week of storage. This suggests the possibility that still other strains of TMV may persist and be capable of infecting tomato seedlings after longer or shorter periods of storage of seeds collected from diseased tomato fruits than hitherto demonstrated.

Kausche (18) reported the presence of a substance in the seed of *Nicotiana tabacum* var. Samsun which inactivated TMV. This inhibiting principle was found both in infected and healthy plants during the process of ripening of the seeds and their germination. From the experiments reported in this paper it appears that an inhibiting principle is present also in tomato seeds. There is no evidence, however, that an extract of germinating seeds is entirely comparable to the sap of an intact seedling. Since extracts of Jubilee tomatoes failed to retard the virus activity appreciably, it was assumed that the strain associated with internal browning might be retained in seeds of Jubilee tomatoes for longer periods. However, experiments carried out with seeds of diseased Jubilee tomatoes germinated at weekly intervals indicated that the virus was retained for not more than 2 weeks.

## SUMMARY

Internal browning of tomato could not be reproduced in tomato fruits by grafting diseased scions onto healthy stocks.

A strain of TMV associated with internal browning of tomato in New Jersey was retained in tomato seeds for only 1 to 2 weeks, while the ordinary strain of TMV was retained for 27 to 28 days.

The strain associated with internal browning differed slightly from the ordinary strain of TMV and the ribgrass strain.

Extracts of germinating tomato seeds exerted an inhibitory effect on infectivity with TMV.

The Laboratories of the Rockefeller Institute for Medical Research
New York, New York

## LITERATURE CITED

1. Bawden, F. C. 1950. Plant viruses and virus diseases. Ed. 3, revised. 335 pp. Chronica Botanica Co., Waltham, Mass.
2. Bawden, F. C. and N. W. Pirie. 1937. The relationships between liquid crystalline preparations of cucumber viruses 3 and 4 and strains of tobacco mosaic virus. Brit. Jour. Exp. Path. **18**: 275-290.
3. Berkeley, G. H., and G. O. Madden. 1932. Transmission of streak and mosaic diseases of tomato through seed. Scient. Agric. **13**: 194-197.
4. Berkeley, G. H., and G. O. Madden. 1933. Transmission of streak and mosaic diseases of tomato through seed. No. II. Scient. Agric. **13**: 455-457.
5. Chamberlain, E. E., and P. R. Fry. 1950. Influence of method of tomato seed extraction on seed transmission of tobacco-mosaic and tomato-streak. New Zealand Jour. Sci. and Tech. A. **32**(2): 19-23.
6. Chester, K. S. 1936. Separation and analysis of virus

strains by means of precipitin tests. Phytopathology **26**: 778-785.

7. Conover, R. A. 1949. Vascular browning in Dade County, Florida green-wrap tomatoes. U. S. Dept. Agr., Pl. Dis. Rptr. **33**: 283-284.
8. Cook, M. T. 1947. Viruses and virus diseases of plants. 244 pp. Burgess Publ. Co., Minneapolis, Minn.
9. Cox, C. E., and L. O. Weaver. 1950. Internal browning of tomatoes in Maryland. (Abs.) Phytopathology **40**: 870.
10. Doolittle, S. P., and F. S. Beecher. 1937. Seed transmisson of tomato mosaic following the planting of freshly extracted seed. Phytopathology **27**: 800-801.
11. Doolittle, S. P., and F. S. Beecher. 1942. A strain of tobacco-mosaic virus causing a necrosis and shrivelling of tomato foliage. Phytopathology **32**: 986-994.
12. Friedman, B. A. 1949. The occurrence of vascular browning or internal browning in tomatoes from Pennsylvania and New York. U. S. Dept. Agr., Pl. Dis. Rptr. **33**: 484.
13. Haenseler, C. M. 1949. Internal browning of tomatoes in New Jersey. U. S. Dept. Agr., Pl. Dis. Rptr. **33**: 336-337.
14. Holmes, F. O. 1941. A distinctive strain of tobacco-mosaic virus from Plantago. Phytopathology **31**: 1089-1098.
15. Holmes, F. O. 1949. Association of strains of tobacco-mosaic virus with internal browning in tomatoes. U. S. Dept. Agr., Pl. Dis. Rptr. **33**: 338-339.
16. Holmes, F. O. 1950. Internal-browning disease of tomato caused by strains of tobacco-mosaic virus from Plantago. Phytopathology **40**: 487-492.
17. Huffington, J. M. 1951. Internal browning and related ripening disorders of tomatoes. Continental Can Co., Inc., Res. Dept. Bul. 25.
18. Kausche, G. A. 1940. Über eine das Virusprotein inaktivierende Substanz im Samen von Nicotiana tabacum Samsun. Biol. Zentralbl. **60**: 423-438.
19. Kleczkowski, A. 1941. Quantitative studies on the serological reactions of some plant viruses and of a pea nodule bacterium (Rhizobium leguminosarum). Brit. Jour. Exp. Path. **22**: 44-58.
20. Knight, C. A. and G. Oster. 1947. The size of the particles of some strains of tobacco mosaic virus as shown by the electron microscope. Arch. Biochem. **15**: 289-294.
21. van Koot, Y. 1949. Enkele nieuwe gezichtspunten betreffende het virus van het tomaten-mozaiek. Tijdsch. Plantenziekt. **55**: 152-166.
22. Lorenz, O. A., and J. E. Knott. 1942. Studies of graywall of tomato. Proc. Amer. Soc. Hort. Sci. **40**: 445-454.
23. Milbrath, J. A. 1937. An indication of seed transmission of mosaic virus in tomato seed. Phytopathology **27**: 868-869.
24. Purdy, H. A. 1929. Immunologic reactions with tobacco mosaic virus. Jour. Exp. Med. **49**: 919-935.
25. Raychaudhuri, S. P. 1952. Retention of strains of tobacco mosaic virus in tomato seeds. (Abs.) Phytopathology **42**: 114.
26. Stoner, W. N., and W. D. Hogan. 1950. A report of graywall or internal browning of tomato in South Florida. U. S. Dept. Agr., Pl. Dis. Rptr. **34**: 379-380.
27. Valleau, W. D. 1939. Symptoms of yellow ring spot and longevity of the virus in tobacco seed. Phytopathology **29**: 549-551.
28. Weaver, E. P., and W. C. Price. 1952. Differentiation of tobacco mosaic virus strains by complement fixation. Proc. Soc. Exp. Biol. Med. **79**: 125-127.
29. Young, P. A. 1946. Tomato diseases in Texas. Texas Agr. Exp. Sta., Circ. 113.

# A NEW SCLEROSPORA ON DICHANTHIUM ANNULATUM [1]

M. J. Thirumalachar and M. J. Narasimhan

Studies on species of *Sclerospora* have evoked interest because of the severe damage incited in cereal crops and forage grasses in restricted areas. Investigations by Weston (1) on species of *Sclerospora* affecting maize and sugarcane have shown their infection potentialities. Weston (3, 4) pointed out the importance of studying *Sclerospora* spp. that occur on wild gramineous plants, since the latter may be collateral hosts for a pathogen occurring on cultivated crops.

From August to December, a *Sclerospora* species is found in Bihar (India) severely affecting the culms of *Dichanthium annulatum* Stapf. a common forage grass in the plains, belonging to the tribe Andropogoneae. Apart from the general interest in studying a species of *Sclerospora* occurring on a new and unrecorded host, the possibility of its connection with downy mildew of maize, caused by *Sclerospora philippinensis* Weston which commonly occurs in the same tract, merited consideration. Appearance of downy mildew on *D. annulatum* coincides with the harvesting of the maize crop; thus, if the same pathogen affects both hosts, the oversummering of *S. philippinensis*, for which no resting spore stage is known, on *D. annulatum* appeared to be a possibility. Detailed morphological and cross inoculation studies, however, revealed that the *Sclerospora* species on *D. annulatum* is distinct from that on maize (*S. philippinensis*) and sorghum (*S. sorghi* (Kulkarni) Weston & Uppal.). A brief account of these studies is presented here.

EFFECT ON THE HOST.—In infected culms, leaves are pale yellow, upturned, and show a white downy growth (remnants of the conidiophores) on the lower surface. In some fields infection was so severe that counts of the diseased culms taken in 100 sq. ft. blocks selected at random showed incidence of infection ranging between 40 and 60 per cent. Later in the season, the diseased plants form inflorescences in the same way healthy plants do. Although the mycelium of the fungus is systemic, there is no incitement of proliferation of the spikelets nor other malformation as observed in the case of infection by other *Sclerospora* species.

CHARACTERISTICS OF THE FUNGUS.—Only the conidial stage of the fungus is known; all attempts to find the oospore stages have been unsuccessful. Field studies indicated that the fungus may oversummer within underground portions of the culms. *Dichanthium annulatum* is a perennial grass which remains green during summer when most other grasses dry up. Soon after the first appearance of disease symptoms in the field in a low lying patch, 25 diseased culms selected at random were carefully dug and their shoots above ground level cut. Underground culm portions with roots attached were transferred to pots and watched for development of new shoots. New shoots showed the paling leaves and downy growth of conidiophores, even from the initiation of the first leaf above ground, thereby indicating that infection is systemic, the mycelium remaining in underground portions of the culms.

As in other species of *Sclerospora* so far known, the conidial phase of the fungus is produced only nocturnally. For studying the conidial stage, infected culms were collected in the evening, atomized with water and inclosed in wide-mouthed bottles that were innerlined with wet paper toweling. Nocturnal observations made at hourly intervals during the middle of September when the nights were humid, indicated that even though there was a heavy condensation of dew drops on the leaves no conidia were formed. Further study indicated that the conidia were produced only when the minimum night temperature reached 70° F. or below. This low nocturnal temperature appeared to be an important factor governing conidial formation and perhaps explains the heavy infection observed in the field during November and December. Weston (2), who made detailed studies on the nocturnal production of conidia in species of *Sclerospora*, stresses only the importance of moisture condensation on the diseased shoots.

Material showing abundant development of conidial stages was sectioned and studied in stained preparations mounted in lactophenol and made permanent by sealing the edge of the coverglasses first with gum-dammar bees-wax mixture and later with thick Canada balsam in xylol. The various stages in development of conidiophores and conidia were followed.

The hyphae are intercellular, coenocytic and contorted. Before the formation of conidiophores they group into hyphal knots in the substomal space. The conidiophore arises as a stout club shaped structure, swollen in the middle and tapering at either end. (Fig. 1, A.) Branches of the main axis are well developed and dichotomous, with only 2 to 6 primary branches, each bearing 2 to 3 obconical sterigmata bearing conidia at the tips (Fig. 1, D, E, I and J). In rare cases secondary and tertiary branches were observed to form under excess moisture conditions. Initials of the primary branches arise as branched lobes at the apex of the main axis (Fig. 1, B and C). Beneath the point of origin of the branches, the main axis becomes swollen. There is a single distinct basal cell in each conidiophore which is isodiametric and shows an inconspicuous knob-like structure at the base. Mature conidia are globose to obovoid, hyaline, thin-walled and germinate immediately by the formation of long germ tubes (Fig. 1, F and G). In some cases a small vesicle forms at the tip of the germ tube (Fig. 1, H). Development of more than 1 germ tube per conidium is a common occurrence.

*Inoculation Experiments.*—Cross inoculation tests were carried out to find whether or not the *Sclerospora* species on *Dichanthium annulatum* would infect maize

[1] Accepted for publication July 21, 1952.

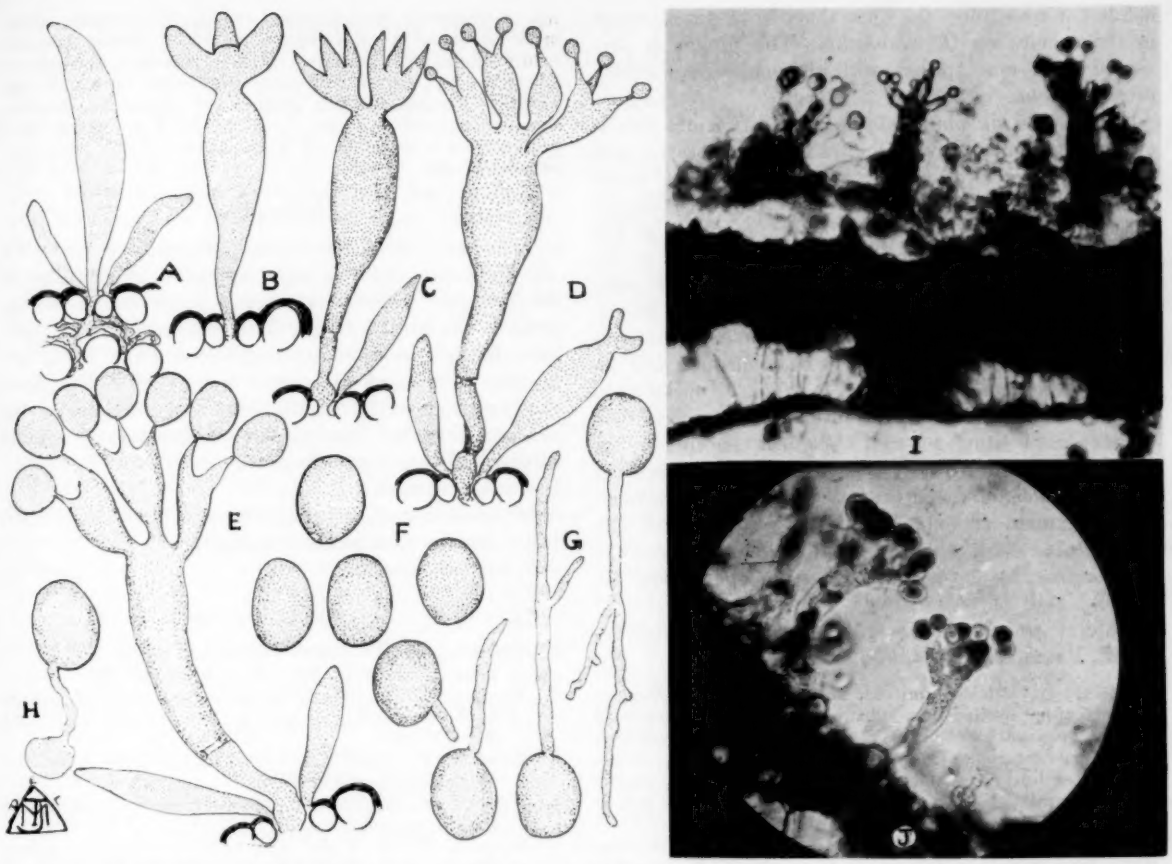



FIG. 1. A) Development of conidiophores × 400. B) and C) Initial stages of conidiophore branches × 400. D) and E) Formation of conidia on sterigmata × 400. F) Mature conidia × 600. G) Germination of conidia × 600. H) Formation of vesicle at the tip of the germ tube × 600. I) Photomicrograph of early stages of conidial formation × 170. J) Conidiophores and conidia × 170.

and sorghum. Sorghum was included in the inoculation studies despite the fact that *Sclerospora sorghi* has not been reported in the north-eastern India. A heavy, downy growth of conidiophores bearing conidia was secured by incubating infected shoots of *D. annulatum* in moist chambers during the early morning hours. An infected leaf with the downy growth was carefully detached from the plant and placed on the young unfolding leaves of maize and sorghum seedlings which had been previously atomized with water, the ventral surface of the leaf bearing conidiophores and conidia was brought into contact with the leaf surface of the plant to be inoculated. Inoculated plants were incubated in moist chambers for 24 hours, after which they were placed outside for further observations. Twenty seedlings of maize and 20 of sorghum were inoculated while 20 noninoculated plants served as controls. Twenty *D. annulatum* plants taken from healthy culms and grown in pots were inoculated to show the infectivity of the fungus.

Observations taken after 10 days indicated that in 12 of the inoculated plants of *D. annulatum* pale yellow streaks and the characteristic downy growth of the fungus appeared. In none of the inoculated plants of maize and sorghum were there signs of infection. Results of cross-inoculation experiments thus confirmed the findings of morphological studies in showing that the *Sclerospora* occurring on *D. annulatum* is not the fungus that incites downy mildews of maize or sorghum.

*Identity of the fungus.*—From comparison of the *Sclerospora* species under study with other species so far known on cultivated crops and wild grasses it is concluded that the former represents a previously undescribed species. The conidial stages of the species on *D. annulatum* are very similar to those of *Sclerospora noblei* Weston described by Weston (4, 5) from Australia on *Andropogon australis* Spreng. (*Sorghum plumosum* Beauv.). *S. noblei* produces both conidial and oosporic stages in succession and Weston points out its endemic nature. The species on *D. annulatum* differs from other species, including *S. noblei*, in size of the conidiophores and conidia, and, chiefly, in possessing a reduced type of conidiophore branching system. Unlike other species of *Sclerospora*, the sterigmata are borne directly on the primary branches of the main axis. Such reduced branching has been reported in some species of *Sclerospora* as an abnormal-

ity, but it constitutes the normal mode of development in the species on *D. annulatum*. The fungus is presented as a new species with the name *Sclerospora dichanthicola*.

*Sclerospora dichanthicola* sp. nov.—The infection is systemic, culmicolous and incites leaf discoloration and development of white mildewy growth on the lower leaf surface. Conidiophores always produced during the night, growing out of the stomata, erect, 83 to 130 $\mu$. long, bearing single basal cells, 13 $\mu$. broad at the septum of the basal cell, 17 to 27 $\mu$. broad at the point of branching of the main axis; basal cell is isodiametric, 33 $\mu$. long 13 $\mu$. broad with an inconspicuous knob-like structure at the base. Branches are well developed, dichotomous, 33 to 37 $\mu$. high, 83 to 90 $\mu$. in spread, usually with primary branches only, 2 to 6 in number, each bearing 2 to 3 obconical tapering sterigmata with conidia, rarely with the main axis showing primary, secondary and tertiary branches before bearing sterigmata and conidia. Mature conidia are globose to obovoid, hyaline, thin-walled, 21-28 $\times$ 15-18 $\mu$., germinating by germ tubes. Resting spores are unknown.

Habitat: in the culms of *Dichanthium annulatum* Stapf., Patna, Bihar, Dec. 18, 1951.

Infectio systemica, culmicola, inducens foliorum discolorationem atque evolutionem substantiae albae rubiginosae in inferiore pagina foliorum. Conidiophori semper noctu producti, emergentes ex stomatibus erecti, 83-130 $\mu$. longi, singulis cellulis basalibus ornati; 13 $\mu$. lati ad septum cellulae basalis, 17-27 $\mu$. lati ad ramificationem axis principalis; cellulae basales isodiametricae, 33 $\mu$. longae, 13 $\mu$. latae, ad basim ornatae structura inconspicua tuberosa. Rami bene evoluti dichotomi, 33-37 $\mu$. alti, 83-90 $\mu$. patentes, ut plurimum rami primarii tantum, 2-6 numero, sterigmata obconica atque fastigata monstrant ornata conidiis, raro producit axis principalis ramos primarios, secundarios vel tertiarios producit antequam sterigmata vel conidia appareant. Conidia matura globosa vel obovoidea, hyalina, tenuibus parietibus ornata, 21-28 $\times$ 15-18 $\mu$.; germinantia per germinationis tubos. Hab. in culmis et foliis *Dichanthii annulati* Stapf.


## SUMMARY

This paper describes a new species of *Sclerospora* (*S. dichanthicola*) inciting a downy mildew disease of *Dichanthium annulatum* Stapf., a perennial forage grass in the Indian plains. The conidial phase is dominant in the life-cycle, resting spore stage being unknown. Compared with other species of *Sclerospora*, the branching of the conidiophore appears reduced, the primary branches bearing the sterigmata and conidia directly. The conidia invariably germinate by formation of germ tubes.

In cross-inoculation experiments *S. dichanthicola* failed to infect maize and sorghum.


BANGALORE, INDIA.

## LITERATURE CITED


1. WESTON, W. H. 1920. Philippine downy mildew of maize. Jour. Agr. Res. (U. S.) **19**: 97-122.
2. WESTON, W. H. 1924. Nocturnal production of conidia by Sclerospora graminicola. Jour. Agr. Res. (U. S.) **27**: 771-784.
3. WESTON, W. H. 1929. A new Sclerospora from Fiji. Phytopathology **19**: 961-967.
4. WESTON, W. H. 1929. A new Sclerospora from Australia. Phytopathology **19**: 1107-1115.
5. WESTON, W. H. 1942. The conidial phase of Sclerospora noblei. Phytopathology **32**: 206-213.

# EXAMINATION OF THE POSSIBILITY OF THERAPY OF PLANT DISEASE WITH IONIZING RADIATION [1]

Paul E. Waggoner and A. E. Dimond [2]

A continuing interest in the therapy of plant diseases led to attempts to cure a plant disease by ionizing radiation. In addition to evaluating plant radiotherapy, these studies provided unique information concerning the nature of disease.

The source of ionizing radiation being used for therapy can be either external or internal in relation to the host. If the diseased plant is merely subjected to ionizing radiation from a nearby source, such as an X ray tube, the source of radiation is external to the plant. In such a case, the radiation must be sufficiently penetrating that all diseased portions of the plant are exposed. When an external source is used, the host and parasite are subjected to similar dosages of radiation, and, if therapy is to be possible, the parasite must be considerably more sensitive to radiation than the host. In the first portion of the present paper, the susceptibility of *Fusarium oxysporum* f. *lycopersici* and of its host, the tomato plant, to gamma and X radiation are examined, a prediction is made concerning the possibility of therapy of Fusarium wilt of tomato, and the prediction is tested.

When the plant is fed a radioactive isotope, the source of radiation is internal, i.e., within the host. If the radioisotope accumulates in the diseased portion of the host, the parasite would be subjected to considerably more radiation than the host receives, especially when soft (i.e., non-penetrating) radiation is used. If accumulation by diseased portions of the plant is sufficiently high, it may be possible to kill the parasite even when it is less sensitive to radiation than is the host. The second portion of the present paper deals with the distribution of radiophosphorus in Fusarium wilted tomato plants and discusses the possibility of therapy of Fusarium wilt when internal sources of radiation are used.

THERAPEUTIC INVESTIGATIONS USING AN EXTERNAL SOURCE OF RADIATION[2].—*Sensitivity of the pathogen, Fusarium oxysporum* f. *lycopersici, to gamma and X rays.*—Cultures of *Fusarium* in test tubes were exposed for varying periods of time to gamma radiation of 25.3 roentgens/hr. intensity from a 20 curie source of $Co^{60}$. Following irradiation, spore suspensions were prepared in a nutrient, and after incubation, the mortality was estimated by counting those spores not germinating. After correcting for natural mortality of spores, the percentage mortality resulting from gamma radiation was estimated as follows: 910 r (roentgens), 0 per cent; 1820 r, 2 per cent; 3640 r, 3 per cent; 7660 r, 6 per cent; 17800 r, 8 per cent; 31500 r, 11 per cent. Each percentage is based upon a count of at least 800 spores. When these data were plotted as a dosage response curve, it was estimated that half the spores would be killed by a dosage of $6.6 \times 10^6$ r.

Susceptibility of *Fusarium* cells to 62 kilovolts X rays (5 milliamperes) was determined by a slightly different technique. In this case, *Fusarium* bud cells were irradiated. *Fusarium* reproduces by budding when grown in liquid medium on a shaker. After irradiation, the cells were placed in fresh nutrient and their ability to germinate was measured. Counts of at least 500 bud cells for each dosage of radiation resulted in the following dosage-mortality data. Counts were corrected for natural mortality. At 0 r, there was 0 percent mortality; 29,000 r, 28 percent; 58,000 r, 23 percent; 86,000 r, 38 per cent; 140,000 r, 55 percent; 200,000 r, 58 percent; 260,000 r, 75 percent. When these data were plotted as a dosage response curve, it was estimated that half the cells would be killed by 125,000 r.

Data on both gamma rays and X rays indicate that more than $10^6$ r are required to eliminate either spores or vegetative cells of the fungus. This means that the host must be extremely resistant to radiation injury if therapy by external sources of radiation is to be possible.

*Sensitivity of the tomato plant to gamma and X rays.*—John Baer tomato plants were exposed to gamma radiation from the 20 curie source of $Co^{60}$. Vegetative growth of plants receiving as much as 5400 r in 36 hours or as little as 3800 r in a month was not affected visibly. Plants exposed to 9600 r during flower set produced heavier vines and less fruit than plants receiving smaller doses. At a radiation intensity of 150 r/hr., growth was suppressed and plants became chlorotic when exposed to from 20,000 to 50,000 r. On being removed from the radiation field, plants recovered. Plants receiving 50,000 r recovered slowly. Plants receiving 100,000 r died. Evidently the maximum dose of radiation which tomato plants will tolerate is of the order of 50,000 r.

Susceptibility of tomato plants to X rays has been examined by Waggoner and Dimond (11); at an intensity of 18,000 r/hr. damage was severe in plants receiving 20,000 r.

*The effect of an external radiation source upon the development of Fusarium wilt.*—From the data so far presented, we can predict the possibility of effecting therapy of Fusarium wilt of tomato by external sources of ionizing radiation. Doses greater than $10^6$ r are required to eliminate either spores or vegetative cells

[1]Accepted for publication July 14, 1952.

Research conducted under contract AT-30-1-(580) between the Atomic Energy Commission and The Connecticut Agricultural Experiment Station, New Haven, Connecticut.

[2]The authors are indebted to the Brookhaven National Laboratory for the use of facilities in the radiation field and to the staff of the Biology Department for help in conducting experiments involving exposure to gamma radiation. They are also indebted to the Biophysics Division of Yale University for use of their facilities in making exposures of plants to X rays.

of the parasite *in vitro*. The host will not survive a dose of $10^5$ r. These statements apply for X radiation at 20 to 30 thousand r/hr. or gamma radiation at 25 r/hr. If, in the diseased plant, the parasite has the same radiation sensitivity as it does *in vitro*, therapy is unlikely; *Fusarium* should survive doses that destroy the host.

The above prediction concerning therapy of wilt was tested by exposing John Baer tomato plants to gamma radiation in the radiation field at the Brookhaven National Laboratory. The plants were inoculated just before they were placed in the field by dipping the roots in a suspension of *Fusarium* bud cells. Noninoculated checks were also exposed.

Seven and a half weeks after inoculation, all plants were graded for Fusarium wilt by estimating the percentage of the primary axis of each plant showing wilting symptoms. (Table 1).

TABLE 1.—*Effect of gamma radiation upon Fusarium wilt of tomato*

| Intensity of radiation r/hr. | Dosage r | Percentage of primary axis with wilt symptoms | Number of plants |
|---|---|---|---|
| 300 | 383,000 | 100 | 2 |
| 150 | 191,900 | 100 | 3 |
| 5.5 | 7,030 | 1.5[a] | 1 |
| 1.6 | 2,050 | 97 | 2 |
| 0.6 | 767 | 100 | 3 |
| 0.4 | 500 | 68 | 4 |
| 0.2 | 281 | 86 | 5 |
| 0.1 | 140 | 66 | 7 |
| 0.05 | 64 | 60 | 10 |

[a]Noninoculated check plants received a grade of less than 3 percent; evidently inoculation was unsuccessful for the plant receiving 7,030 r.

Results of exposure of inoculated plants to gamma radiation confirm the earlier prediction: ionizing radiation from an external source is not a successful therapeutic agent because the host is damaged by lower doses than is the parasite. It even appears that disease severity is increased at the higher dosages.

If radiation does lead to increased disease severity, an interesting hypothesis for the mode of action can be constructed from the following observations: first, X radiation is known to inactivate auxin (6). Second, Davis (4) has found that indole acetic acid decreases the severity of tomato wilt. It follows that the increase in severity of symptoms may be due to inactivation of auxin by X radiation. This aspect is under study.

THERAPEUTIC INVESTIGATIONS USING AN INTERNAL SOURCE OF RADIATION.—Evidently the differential susceptibility of host and parasite to ionizing radiation does not permit elimination of the parasite and survival of the host when both receive the same dose. Consequently, therapy of Fusarium wilt by an internal source of radiation will be possible only if the parasite receives a larger dose than the host because the source, a radioactive isotope, accumulates in the vicinity of the parasite. The accumulation must be great enough to offset the unfavorable difference between host and parasite in radiation susceptibility. Distribution of a radioisotope in diseased tomato plants is easily measured. Thus it can be determined whether or not sufficient accumulation occurs in diseased tissues to provide for the possibility of therapy by an internal source of radiation.

With vascular wilt diseases of plants there are 2 mechanisms which can result in an accumulation of a radioisotope in the region invaded by the parasite: metabolic accumulation and vascular stoppage. The first of these is known to occur in a number of instances. Thus, accumulation of radiophosphorus in or near fungi growing on plants has been demonstrated by radioautographic techniques for rust on grasses (7) and mycorrhizal fungi on roots of pine seedlings (8). Similarly, radio-sulfur accumulates in the vicinity of rust or mildew infections on bean (12). These are instances of metabolic accumulation originating from a more rapid turnover of compounds in the region of infection than in the uninfected portions of the host. Because $P^{32}$ is known to be accumulated in the region invaded by the parasite and because its counting characteristics are convenient, $P^{32}$ was chosen as the isotope for use in this study.

Vascular stoppage might be an important factor in accumulation of isotopes in the infected region of the host attacked by a vascular wilt disease. Thus, $Rb^{86}$ uptake was restricted in oak trees showing symptoms of oak wilt (1), indicating that radioisotopes in the xylem can be prevented from passing into parts of the host above the diseased portion. Fusarium wilt of tomato causes a similar vascular dysfunction (9, 10). Hence, if vascular dysfunction confines a radioisotope to infected xylem, the radiation levels will be higher in this area than in healthy portions of the plant.

It is uncertain whether a radioisotope will be completely confined through vascular dysfunction to the infected region of the host. Scheffer (10) succeeded in moving dyes through infected vessels when force was applied for a long time. Moreover, the radioisotope may by-pass vessels which conduct the transpiration stream poorly and be moved to the growing point either through unimpaired vessels or through phloem. Thus $P^{32}$ was found to move from roots through phloem to the upper shoot, although the xylem was the primary avenue of movement (3). When $P^{32}$ is applied to host roots for an extended period, as it must be for therapeutic purposes, and if wilt-induced vascular dysfunction is limited to the xylem, some $P^{32}$ will pass readily into leaves and growing point unless the isotope is concentrated by metabolic accumulation. The accumulation must prevent most of the isotope from reaching the growing point if that radiation-sensitive part (2) is not to be injured.

*Distribution of $P^{32}$ in diseased plants.*—To determine the distribution of $P^{32}$ in diseased plants, Bonny Best tomatoes were grown in sand to which nutrient was added 3 times a week. When the plants had 4

TABLE 2.—*Distribution of radiophosphate in healthy and Fusarium wilted tomato plants*

| Days after inoculation | Severity of vascular discoloration in diseased plants[a] | Radiophosphate content of tomato tissue (Counts per sec. per mg.) | | | | | |
|---|---|---|---|---|---|---|---|
| | | Growing point | | Top 4 leaves | | Top 4 internodes | |
| | | Inoc. | check | Inoc. | check | Inoc. | check |
| 12 | 1.3 | 42.7 | 46.8 | 27.8 | 21.9 | 28.5 | 20.0 |
| 17 | 4.6 | 22.4 | 40.8 | 10.8 | 17.0 | 12.8 | 13.4 |
| 22 | 7.5 | 7.9 | 11.0 | 4.9 | 5.8 | 6.4 | 5.1 |

[a] The vascular discoloration score ranges from 0 for no discoloration and 1 for 1 bundle slightly discolored to 3 × 6 or 18 for 6 bundles severely discolored in 1 internode.

true leaves, 15 were inoculated by dipping the roots in a bud cell suspension of *Fusarium*, produced in a shake culture. Fifteen noninoculated plants were transplanted at the same time. Ten, 15, and 20 days after inoculation about 1/50 cm. of $H_3P^{32}O_4$ in 50 ml. of balanced nutrient solution was used to fertilize each of 5 noninoculated and five check plants. Two days after radiophosphate was applied, plants were examined. Examination of the plants consisted of measuring height and weight, scoring the leaves and vascular bundles for wilt symptoms (5), and determining[3] the radioactivity of the growing point and certain leaves and internodes.

Results in Table 2 demonstrate clearly that $P^{32}$ was generally distributed throughout diseased plants as it was in healthy ones. Accumulation of radiophosphorus in parasitized portions of the plant did not greatly decrease the radiophosphorus in the growing point and upper leaves which were not yet invaded by the parasite. Consequently, it is unlikely that *Fusarium* can be inactivated by $P^{32}$ as an internal source of ionizing radiation without damaging the host.

Although $P^{32}$ was not greatly decreased in the upper parts of the plants, and therapy is unlikely, the uptake by the growing point was frequently slightly decreased by the presence of *Fusarium*. Uptake was decreased significantly after 17 days and non-significantly after 12 and 22 days in the case of the inoculated plants described in Table 2. The decrease in phosphorus uptake on the twenty-second day was ascribed to environmental conditions and plant age because it occurred in both diseased and healthy plants. In repeated experiments these same results were obtained. Whether readings on plants were taken after only 1 day's exposure to $P^{32}$ or after 3 days' exposure, whether the quantity of radiophosphate was less or greater than 1/50 mc. per plant (maximum dosage was 8 times the minimum dosage), the amounts of radiophosphate in growing points of diseased plants that were examined were frequently slightly less than those in healthy plants. Beckman et al (1) observed that the uptake of $Rb^{86}$ is restricted in wilted oak trees. Evidently, decreased salt uptake is common in pathologically wilted plants.

If the *Fusarium* present in tomato stems accumulated $P^{32}$ much more rapidly than host tissue, the amount of $P^{32}$ should be greater in an infected stem relative to the amount in the leaves than in a healthy plant. The vascular bundles of the stems of the plants, Table 2, inoculated 17 and 22 days previously were severely discolored. This was probably associated with a severe infection of *Fusarium*.[4] Although the effect is not statistically significant, in this sample the amount of $P^{32}$ in stems relative to the amount in leaves is greater in inoculated than in healthy plants. Because the mass of *Fusarium* in the stem is so much smaller than the mass of host tissue, it is not surprising that the amount of $P^{32}$ is not increased significantly. The radioautographic or some other differential technique is required to detect the extent of radiophosphate accumulation by *Fusarium* mycelium in the host.

*Distribution of $P^{32}$ in necrotic stems.*—Evidently, discoloration of the vascular bundles does not greatly restrict the uptake of phosphorus. Evidently, too, water movement is severely curtailed in vessels discolored by the action of *Fusarium* (9, 10). These facts suggest that phosphorus can move through channels in the diseased stem which are not available to water movement, e.g., the phloem (3), or secondary vascular elements which are not discolored. Although these 2 avenues are open to phosphorus and not to water in a stem showing vascular discoloration, they should be closed to phosphorus in a stem which has become generally necrotic.

To examine this possibility, pairs of tomato plants having 2 true leaves were inoculated and planted in a single veneer band. After 15 days $P^{32}$ was applied to the sand. After 76 hours, 2 pairs of check plants and 2 pairs of diseased plants were examined (Table 3). The stem of one plant of each pair of diseased plants had become generally necrotic while the other was not, although the leaves showed symptoms of Fusarium wilt. While no suppression of phosphorus uptake was associated with the moderate wilt symptoms, phosphorus uptake in the necrotic stems was nearly zero (Table 3). Evidently the avenues open to phosphorus uptake

[3] Oven dried samples were weighed and placed under a thin-window Geiger tube. The resulting counts corrected for amount of $P^{32}$ to which the plants were exposed, for decay, and for background were expressed as the counts per second per mg. of tissue. Constant self absorption was assumed. This provided numbers proportional to the concentration of $P^{32}$.

[4] Other studies in this laboratory have shown that the number of bundles infected by *Fusarium* is roughly proportional to the vascular discoloration score even when vascular discoloration is increased by maleic hydrazide application.

TABLE 3.—*$P^{32}$ content of growing point and youngest 3 leaves of healthy, moderately diseased and necrotic tomato stems.*

| Pair No. | Discolored stem | Necrotic stem |
|---|---|---|
| I | 89.1[a] | 0.40 |
| II | 19.1 | 0.14 |
| | Healthy stems | |
| III | 25.1 | 75.9 |
| IV | 39.8 | 89.1 |

[a] Counts per sec. per mg.

in a stem whose vascular bundles are discolored by *Fusarium* are closed in a stem that has become generally necrotic.

## SUMMARY

The conditions necessary for therapy of a plant disease by ionizing radiation were examined. Using Fusarium wilt of tomato as a model, the sensitivity of *Fusarium oxysporum* f. *lycopersici* and of the tomato plant to ionizing radiation were determined. Because the pathogen, *Fusarium*, is considerably more resistant to ionizing radiation than the host, it was predicted that therapy of Fusarium wilt could not be effected with a source of radiation external to the host. This prediction was verified by experiments using gamma radiation from $Co^{60}$.

Radioisotopes could possibly be accumulated in diseased tissue as a result of metabolic accumulation or as a result of mechanical stoppage of translocative processes. Because of such effects, therapy might have been possible when a radioisotope was used which was accumulated in diseased tissues to such a degree that death of the parasite was favored while injury to the host was minimized. To examine this possibility the distribution of radiophosphate was measured in healthy and diseased tomato plants. It was learned that the amount of radioactivity arriving at the growing point of a diseased plant was only slightly less than in the case of a healthy plant. Therefore, therapy of Fusarium wilt by internal sources of radiation seems unlikely.

Stems which had become necrotic from the effects of *Fusarium* no longer translocated appreciable amounts of phosphate to leaves and growing point. However, stems which were discolored translocated about as much phosphate to growing point and terminal leaves as healthy tomato stems.

DEPARTMENT OF PLANT PATHOLOGY
THE CONNECTICUT AGRICULTURAL EXPERIMENT STATION
NEW HAVEN, CONNECTICUT

## LITERATURE CITED

1. BECKMAN, C. H., J. E. KUNTZ, A. J. RIKER, AND J. G. BERBEE. 1952. Plugging of vessels associated with oak wilt development. (Abs.) Phytopathology **42**: 2.
2. BLUME, J. M., C. E. HAGEN, AND R. W. MACKIE. 1950. Radiation injury to plants grown in nutrient solutions containing $P^{32}$. Soil Sci. **70**: 415-426.
3. CHEN, S. L. 1951. Simultaneous movement of $P^{32}$ and $C^{14}$ in opposite directions in phloem tissue. Amer. Jour. Bot. **38**: 203-211.
4. DAVIS, D. 1952. Inducing disease resistance with plant growth regulators. (Abs.) Phytopathology **42**: 465.
5. DIMOND, A. E., D. DAVIS, R. A. CHAPMAN, AND E. M. STODDARD. 1952. Plant chemotherapy as evaluated by the Fusarium wilt assay on tomatoes. Conn. Agr. Exp. Station Bulletin 557.
6. GORDON, S. A., AND R. P. WEBER. 1950. The effect of X radiation on indoleacetic acid and auxin levels in the plant. (Abs.) Amer. Jour. Bot. **37**: 678.
7. GOTTLIEB, D., AND J. M. GARNER. 1946. Rust and phosphorus distribution in wheat leaves. Phytopathology **36**: 557-564.
8. KRAMER, P. J. AND K. M. WILBUR. 1949. Absorption of radioactive phosphorus by mycorrhizal roots of pine. Science **110**: 8-9.
9. LUDWIG, R. A. 1952. Studies on the physiology of hydromycotic wilting in the tomato plant. Macdonald College. Tech. Bul. In press.
10. SCHEFFER, R. P. 1952. The wilting mechanism in Fusarium wilt of tomato. (Abs.) Phytopathology **42**: 18.
11. WAGGONER, P. E. AND A. E. DIMOND. 1952. Crown gall suppression by ionizing radiation. Amer. Jour. Bot. In press.
12. YARWOOD, C. E. AND L. JACOBSON. 1950. Selective absorption of sulphur-35 by fungus-infected leaves. Nature **165**: 973.

# ADAXIAL CRACK STEM OF CELERY [1]

John T. Middleton, Bernarr J. Hall, R. T. Wedding, and J. B. Kendrick, Jr.

A brown discoloration, corking, and checking of the adaxial petiole surface of celery, *Apium graveolens* L., has been observed in plantings in coastal California since 1945. The disease has become increasingly important, especially in fall, winter, and early spring plantings, with losses varying from less than 1 to more than 90 per cent. It is the purpose of this report to describe the symptoms of the disease, the inciting agent, and control measures.

SYMPTOMS OF THE DISEASE.—The imbricated, incompletely green petioles of affected plants first show a watersoaked, somewhat elongated area about 1 to 2 mm. wide and 2 to 5 mm. long, on the distal portion of the adaxial surface (Figure 1, A). As the petioles elongate and become green the watersoaked area increases in length, assumes a wavy margin, and turns yellow-brown (Figure 1, B). Some of the affected tissue later becomes necrotic and margins appear etched and dark brown or black. Continued elongation of the petioles produces transverse checks and cracks, and the affected area appears corky (Figure 1, C). Similar but smaller lesions occasionally appear on the adaxial surface of the petiolules (Figure 1, D). Lesions on mature petioles may ultimately vary in length from 2 to 20 cm. and in width from 5 to 20 mm. Petioles of severely diseased plants are deeply cupped with margins touching or separated by only a few millimeters. Sometimes the tissue above and adjacent to the vascular bundles is etched and corky (Figure 1, C).

Celery plants affected with adaxial crack stem are typically not stunted and have normal pith and laminas. In this respect they differ markedly from celery affected with abaxial crack stem induced by boron deficiency as reported by Purvis and Ruprecht (7). Adaxial crack stem occurs most frequently in celery plantings which have received abundant amounts of

[1]Accepted for publication July 14, 1952.

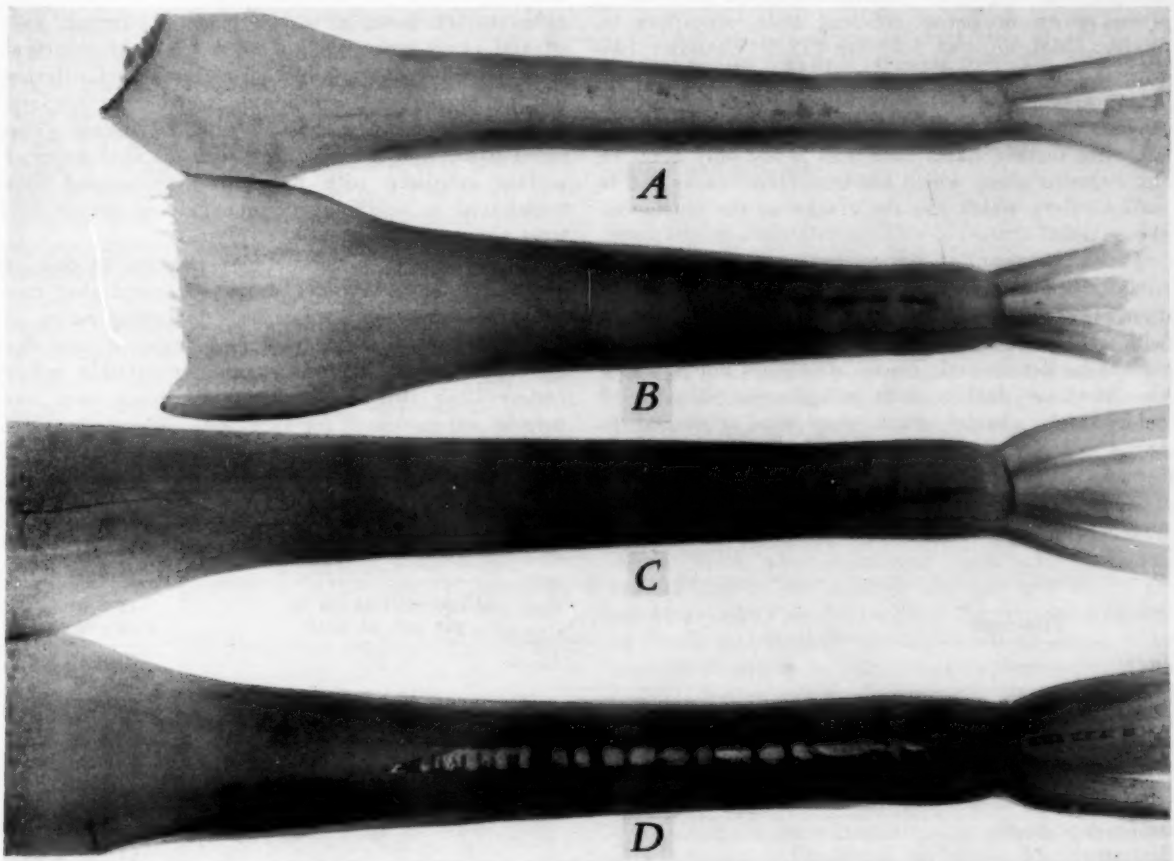



FIG. 1. Petiole and petiolule symptoms of adaxial crack stem of celery. A) Initial watersoaked area. B) Enlarged area with wavy margin and initiation of necrotic lesion. C) Transverse checks and cracks with corky tissue above the vascular bundle. D) Deeply cupped petiole with severe checking and cracking together with similar symptom on the petiolules.

nitrogenous fertilizers, both inorganic and organic. These applications often shorten the growth period to harvest by increasing plant growth rate and in so doing accentuate plant deficiencies of the micronutrients. The disease appears most commonly on the variety Utah 10B, somewhat less frequently on Utah 16, but has not been observed on Summer Pascal.

The similarity of the adaxial petiole symptom in celery to that induced by boron deficiency in other crops suggests that this disease may also be induced by boron deficiency, although not previously ascribed to it. Walker, Jolivette, and McLean (11) describe a cross hatching of the adaxial surface of the petiole of garden beet, *Beta vulgaris* L., as a leaf symptom of boron deficiency associated with internal black spot; they report a similar expression on petioles of sugar beet leaves, *B. vulgaris* var. *macrorhiza* Stev. Walker and Hare (10) observed cross hatching, browning, and corking of the adaxial surface of chard, *B. vulgaris* var. *cicla* L., leaves on plants grown in boron deficient soil; this condition was largely controlled by the addition of boron to the plants or soil. Leaves of cabbage, *Brassica oleracea* var. *capitata* L., and leaves of cauliflower, *B. oleracea* var. *botrytis* L., exhibit corking and checking on the adaxial surface of the petioles when grown in boron deficient soils, according to Walker, McLean, and Jolivette (12). Chandler (4) reports that leaves of chinese cabbage, *B. pekinensis* Rupr., display transverse cracks on the adaxial petiole surface when grown in a boron deficient culture medium. He further states that "this is the only plant in the *Brassica* group which has transverse cracks and is unlike celery which has the cracks on the outside of the petiole."

Foster (5) applied the term "brown stem" to an undescribed nonparasitic disease of celery reported in Florida in 1924. Whether brown stem is identical with or related to adaxial crack stem in California cannot be determined; brown stem does not figure in the literature dealing with nonparasitic diseases of celery after abaxial crack stem was attributed to boron deficiency. Smith and Ramsey (9) describe a brown spot of petioles and laminas of celery which is incited by the fungus *Cephalosporium apii* Smith and Ramsey. Segall (8) reports the occurrence of this same disease in New York on green Pascal celery. The symptoms depicted by Smith and Ramsey in their Figure 1, D, G, and that of Segall's Figure 1 are indeed similar to the adaxial crack stem disease now occurring in California. Bardin (1) was the first to report abaxial crack stem from Salinas, California, and to note the corking and checking of the adaxial petiole surface of celery, and further to associate this condition with apical bud failure. He attributed abaxial crack stem to boron deficiency and suggested that bud failure and adaxial corking might be associated with this deficiency. Bardin later (2) observed that bud failure was not associated with either abaxial or adaxial crack stem and that applications of boron to the soil adjacent to immature celery plants failed to produce conclusive evidence that the disorder was related to boron deficiency. Bardin and McCormick (3) reported that application of boron salts to soil reduced the occurrence of crack stem in a single trial. Middleton, Kendrick, and Hall (6) recently reported the occurrence of adaxial crack stem in southern California and suggested that the disease was incited by boron deficiency.

MATERIALS AND METHODS.—Celery affected with adaxial crack stem was collected from the principal celery growing districts of coastal southern California for the purpose of determining whether or not any plant pathogen was associated with the disease. Diseased tissue was either washed with distilled water or surface sterilized with mercuric cyanide and then transferred to acidified and nonacidified potato dextrose agar, and cornmeal agar plates.

The similarity of the petiole symptom to that of boron deficiency in other crops suggested that controlled micronutrient spray tests including boron as well as copper, iron, magnesium, manganese, and zinc be conducted. Plantings of the susceptible celery variety Utah 10B in which adaxial crack stem was present were selected for spraying. Water sprays of the above mentioned materials were applied to 5 replicated plots at the rate of approximately 62 gal. per

TABLE 1.—*Effect of micronutrient spray applications upon the recurrence of adaxial crack stem of celery 45 days after treatment*

| Treatment | Concentration in oz. per gal. of water | Per cent recurrence |
|---|---|---|
| Borax | 2 | 0.0** |
| Untreated | | 48.8 |
| Cuprous oxide | ⅓ | 24.8 |
| Untreated | | 38.8 |
| Ferric ammonium citrate | 1 | 14.8 |
| Untreated | | 55.0 |
| Magnesium sulfate | ⅓ | 0.0* |
| Untreated | | 47.2 |
| Manganese dioxide | ⅓ | 0.0 |
| Untreated | | 54.2 |
| Zinc sulfate | ½ | 14.3 |
| Untreated | | 12.8 |

**Significantly lower than untreated at 99:1 by "t" test.
*Significantly lower than untreated at 19:1 by "t" test.

acre. The materials used and their concentration are in Table 1. Disease development progressed well in only 2 of the plots established. Each plant in these 2 experiments was critically inspected 42 days after spraying in one instance and 45 days later in the other for the presence of the disease on mature petioles as well as on the immature, imbricated petioles of the apical bud which had developed subsequent to treatment.

RESULTS.—The majority of the isolations made from tissue platings of diseased petioles remained sterile. While some platings yielded miscellaneous fungi, in no instance was any single organism consistently recovered.

The effect of micronutrient sprays upon the development of adaxial crack stem of celery treated at Chula Vista, California, on October 30, and observed on December 14, 1951, is presented in Table 1. The results secured February 1, 1952, following treatment at Chula Vista on December 21, 1951, are in Table 2. Both sets of data demonstrate that the application of borax to celery affected with adaxial crack stem arrests the development of the disease. Data also indicate that recurrence of the disease is suppressed by treatment with magnesium sulfate in the first experiment, and by manganese dioxide in the second.

Observations made for the past several years in a large number of celery plantings affected with adaxial crack stem reveal that the disease most frequently appears in the absence of abaxial crack stem symptoms. This is illustrated in Figure 2, A, C, in which both surfaces of the same petiole are depicted. Records of the occurrence of adaxial with abaxial crack stem have been obtained from 2 celery plantations at Chula Vista grown for winter harvest in 1952. The association of these 2 symptoms is presented in Figure 2, B, D, which illustrates both surfaces of the same petiole. The relation of the one symptom with the other has been shown in the data secured from one planting. Statistical analysis of these data shows a highly significant degree of correlation ($r = 0.77$) between the occurrence of the 2 symptoms. This is further evidence that the adaxial petiole disease of celery is incited by boron deficiency.

TABLE 2.—*Effect of micronutrient spray applications upon the recurrence of adaxial crack stem of celery 42 days after treatment*

| Treatment | Per cent recurrence[a] |
|---|---|
| Borax | 0.0* |
| Borax, manganese dioxide, and magnesium sulfate | 0.1* |
| Cuprous oxide | 17.9 |
| Ferric ammonium citrate | 8.8 |
| Magnesium sulfate | 11.0 |
| Manganese dioxide | 7.8* |
| Zinc sulfate | 17.5 |
| Untreated | 29.3 |
| Least significant difference 19:1 | 21.0 |

[a]Analysis of variance performed on per cent recurrence transformed to: angle = arc sin $\sqrt{\text{percentage}}$.
*Significantly lower than untreated at 19:1.

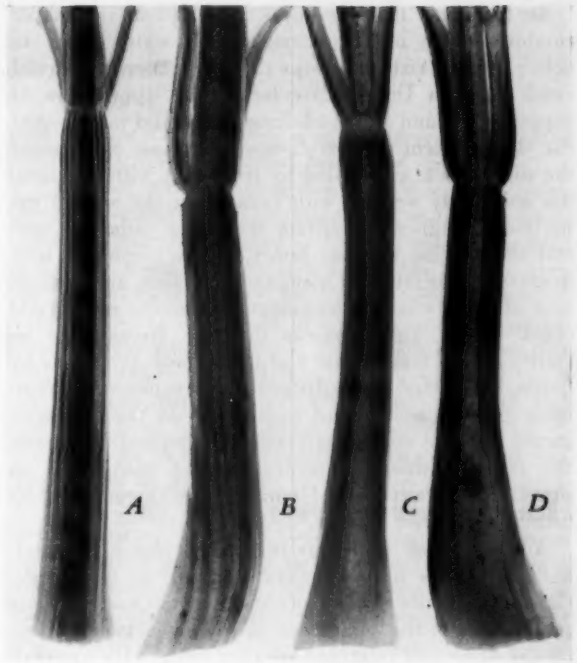



FIG. 2. Adaxial and abaxial crack stem of celery. A) Abaxial surface with no crack stem symptom. B) Abaxial surface with crack stem apparent on petiole and petiolules. C) Adaxial crack stem on the same petiole shown in A. D) Adaxial crack stem on the same petiole shown in B, demonstrating the presence of both symptoms on some petioles and petiolules.

DISCUSSION.—The similarity between the brown spot disease as reported and figured by Segall and adaxial crack stem of celery as presented here is striking, yet no causal fungus has been found associated with the disease in California. Some of the material reported by Smith and Ramsey is likewise very similar to the disease described by the authors, although Smith and Ramsey show their causal fungus to be pathogenic to petioles and laminas of both seedling and mature celery plants, whereas adaxial crack stem symptoms are confined to the petioles, and are only rarely observed on celery seedlings. Since the symptoms figured by Smith and Ramsey are the result of infection by inoculation with pure cultures of *Cephalosporium apii* and no disease symptoms appeared on noninoculated plants, it is apparent that the fungus may also induce adaxial crack stem symptoms on petioles which resemble that caused by boron deficiency. Further evidence that the two diseases are caused by different inciting agents is the occurrence of brown spot on Pascal and Golden Self-Blanching varieties. These varieties are not known to be affected with adaxial crack stem caused by boron deficiency. So far as the writers are aware brown spot of celery has not been found in southern California.

Results show that the application of borax alone or combined with other materials as a water spray to celery foliage virtually stops the recurrence of adaxial crack stem in Utah 10B celery. The application of copper, iron, and zinc as foliate sprays did not prevent the development of the disease. In one experiment the disease was controlled by treatment with magnesium sulfate as well as with borax. In the second experiment magnesium sulfate treatment failed to control the disease whereas borax, borax combined with magnesium sulfate and manganese dioxide, and manganese dioxide effected significant reduction of adaxial crack stem. This suggests that the disease can be controlled by magnesium and manganese as well as by boron. Because of the inconsistent response to treatment by magnesium and manganese in the 2 experiments and the consistently effective control by boron, the possible role of magnesium and manganese in preventing recurrence of adaxial crack stem will be critically studied further.

Adaxial crack stem was responsible for a considerable crop loss in one celery planting at Oceanside, California, while no loss occurred in the same variety planted about the same time in the same location and on the same soil type, but sprayed twice with approximately 10 lbs. of borax per acre.

The disease has sometimes been called "brown checking." Although the symptoms occur only on the adaxial petiole surface, and most typically in the absence of abaxial crack stem, when both symptoms are present the abaxial is typically associated with the adaxial. Since the adaxial symptoms are the first to appear and are usually the only ones evident in celery plantations, and the disease can be controlled by the application of borax, the authors believe that the term "adaxial crack stem" properly shows the relation of the disease to its causal agent and is more descriptive than "brown checking."

It seems probable that the increasing prevalence and importance of this disease are due to the general acceptance and widespread use of the celery variety Utah 10B. A series of low rainfall years may also have been a factor in its increased occurrence.

## SUMMARY

A serious disease of Utah type celery affecting the adaxial surface of petioles and petiolules has been observed in coastal California since 1945. The disease is particularly severe on Utah 10B, especially when grown under extremely high nitrogen fertilization. Diseased tissue first appears watersoaked, later turns dark and becomes necrotic, and ultimately exhibits corky, transverse checks and cracks. The disease is not associated with any plant pathogen, but is induced by boron deficiency. Although the disease typically develops in the absence of abaxial crack stem symptoms, both types of symptom expression may develop on some plants. The disease may be controlled by applying water sprays of borax to celery foliage.

Divisions of Plant Pathology and Plant Physiology
University of California,
Riverside, California
and
University of California Extension Service
College of Agriculture,
San Diego, California

## LITERATURE CITED

1. Bardin, R. 1947. Association of split heart or doubles with crack-stem of celery. U. S. Dept. Agr., Pl. Dis. Reptr. **31**: 119-120.
2. Bardin, R. 1948. Crack-stem of celery in Salinas Valley. U. S. Dept. Agr., Pl. Dis. Reptr. **32**: 15-16.
3. Bardin, R., and J. A. McCormick. 1949. Boron nutrition of celery. Western Grower and Shipper 20(5):20-21.
4. Chandler, F. B. 1940. Boron deficiency symptoms in some plants of the cabbage family. Maine Agr. Expt. Sta. Bul. 402.
5. Foster, A. C. 1924. Florida celery diseases. U. S. Dept. Agr., Pl. Dis. Reptr. **8**: 9-10.
6. Middleton, J. T., J. B. Kendrick, Jr., and B. J. Hall. 1952. Parasitic and nonparasitic diseases of celery in southern California. U. S. Dept. Agr., Pl. Dis. Reptr. **36**: 188-193.
7. Purvis, E. R., and R. W. Ruprecht. 1937. Cracked stem of celery caused by a boron deficiency in the soil. Fla. Agr. Expt. Sta. Bul. 307.
8. Segall, R. 1951. Brown spot disease of celery found in New York. U. S. Dept. Agr., Pl. Dis. Reptr. **35**: 164.
9. Smith, M. A., and G. B. Ramsey. 1951. Brown-spot disease of celery. Bot. Gaz. **112**: 393-400.
10. Walker, J. C., and W. W. Hare. 1944. Boron deficiency studies. *ex.* Boron in Agriculture. Pacific Coast Borax Company.
11. Walker, J. C., J. P. Jolivette, and J. G. McLean. 1943. Boron deficiency in garden and sugar beet. Jour. Agr. Res. [U. S.] **66**: 97-123.
12. Walker, J. C., J. G. McLean, and J. P. Jolivette. 1941. The boron deficiency disease in cabbage. Jour. Agr. Res. [U. S.] **62**: 573-587.

# BLIGHTS OF SNAP BEAN IN GEORGIA [1]

E. S. Luttrell and K. H. Garren

Investigation of snap bean blights in the commercial bean-growing region of South Georgia has shown that the organisms primarily responsible for these diseases are *Macrophomina phaseoli* (Maubl.) Ashby, *Rhizoctonia solani* Kühn, and *Sclerotium rolfsii* Sacc. Since these fungi are commonly associated with important diseases of a number of crop plants, results of field and green house studies on their relationship to diseases of snap bean may be of interest. These species are representative of a group of fungi which may exist as saprophytes in the soil and in which the vegetative mycelium serves as the inoculum.

In this report particular attention has been given to *Macrophomina phaseoli* because of divergent results obtained in previous studies of its pathogenicity on various crop plants and because it causes 2 distinct diseases of snap bean: ashy stem blight in which infection results largely from air-borne conidia, and charcoal rot in which infection is accomplished by mycelium in the soil. The diseases caused by this fungus afford an excellent illustration of the contrast between diseases in which the inoculum is air borne and those in which the inoculum develops in the soil.

THE DISEASES.—*Ashy Stem Blight and Macrophomina Leaf Spot.*—Ashy stem blight and leaf spot caused by *Macrophomina phaseoli* are primarily diseases of mature plants. The leaf spots, as noted previously (10), usually appear first at the tips of leaflets as irregular, brown, dead areas (Fig. 1, A). The lesions are soon covered with tiny black pycnidia. From the margin of the spot a reddish-brown discoloration of the veins, especially conspicuous on the lower surface of the leaf, spreads downward toward the petiole. Ultimately the entire leaf dies and becomes covered with pycnidia. If the infected leaf clings to the plant, the fungus may spread down the petiole and enter the stem.

Ashy stem blight lesions girdle the stems, resulting in a blight of the entire plant. The first symptom of the disease is a reddish-brown discoloration of the stem. The lesions may occur at any point on the stem; but they commonly appear at a node, indicating that the fungus usually enters the stem through infected petioles (Fig. 1, B). When the lesions girdle the stem the distal portions of the shoot wilt and die back (Fig. 1, C, D). The dead stems become pale gray and densely speckled with black, embedded pycnidia. The dried pods are likewise covered with pycnidia, and the seed inside may be rotted and covered with tiny black sclerotia (Fig. 1, E). Ashy stem blight usually appears late in the season after the crop is harvested and has little effect on the production of green beans. As the pods dry, however, infection becomes general. This disease, therefore, would prohibit the growing of dried beans for seed (1).

*Charcoal rot.*—Charcoal rot is a disease of young seedlings caused by *Macrophomina phaseoli.* Entire seedlings may be blighted and blackened during emergence, or elongated black cankers may appear along one side of the hypocotyl after emergence. The cankers almost invariably spread from blackened cotyledons that became infected during emergence and extend up and down the stem from the cotyledonary node. Occasionally cankers extend up the hypocotyl from the soil level. The cankers usually result in the blighting of entire seedlings, but slight lesions may heal. Pycnidia are present on the above-ground lesions. Sclerotia form within the tissues and on the underground parts. In South Georgia bean fields charcoal rot generally appears to be of minor importance, although moderately heavy infections have been observed in some fields, and Harter and Zaumeyer (5) have reported 5 to 50 per cent infection in Georgia plantings.

*Rhizoctonia stem canker and blight.*—Infection with *Rhizoctonia solani* may result in the complete decay of seedlings during emergence, post-emergence damping off, or stem canker of older plants. The symptoms are tan to reddish-brown, sunken lesions spreading up one side of the hypocotyl from the ground level. Infected seedlings may wilt and fall over or fall over and grow prostrate for several days before dying. Cankers may be found on the stems of older plants at the soil level, but the stunted condition of these plants indicates that infection probably occurred in the seedling stage. Apparently infection does not occur after the plants have passed the blooming stage. The perfect stage of *R. solani, Pellicularia filamentosa* (Pat.) Rogers, occurs as a tenuous white coating over the living stems of mature plants. *R. solani* usually does not appear in plantings made in early spring but in later plantings may seriously reduce stands.

*Southern blight.*—Southern blight, caused by *Sclerotium rolfsii,* is characterized by light-colored, shrunken lesions girdling the stem at the soil level and resulting in the wilting and death of the entire plant. The white mycelium of the fungus surrounds the stem and forms its characteristic spherical brown sclerotia. *S. rolfsii* causes pre-emergence and post-emergence damping off as well as blight of older plants. It is commonly present in bean fields, although usually only a few plants in isolated spots are infected. However, during periods of wet, warm weather it may cause considerable damage.

MATERIALS AND METHODS.—The snap bean variety used in all experiments was Tendergreen. For making soil inoculations the fungi isolated from diseased plants were grown in flask cultures on sterilized oats. The oat inoculum was added to 8 in. pots of an autoclaved mixture of compost and soil at the rate of 4

[1]Accepted for publication July 18, 1952.
Paper No. 220, Journal Series, Georgia Experiment Station.

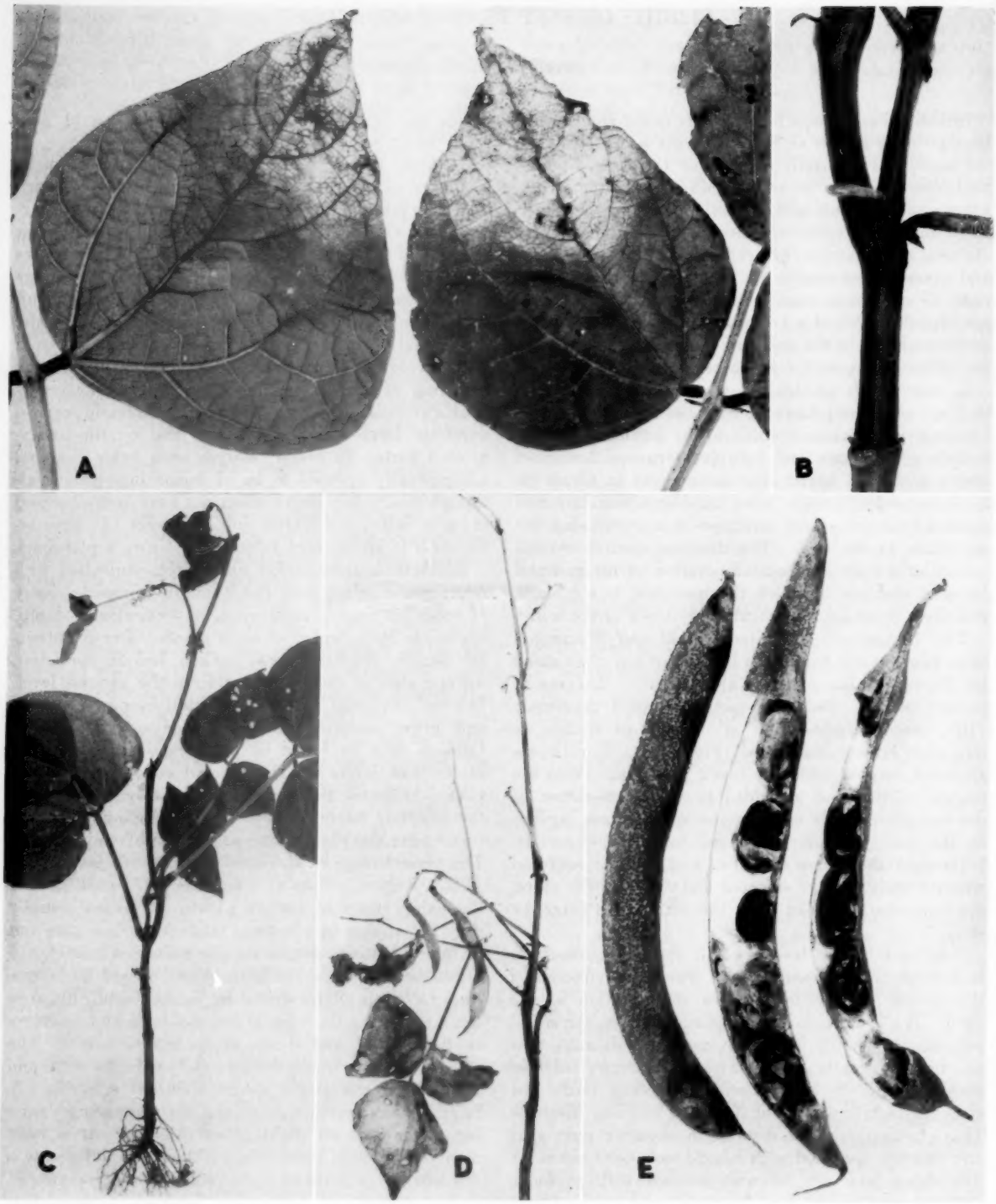



FIG. 1. Ashy stem blight and leaf spot of snap bean caused by *Macrophomina phaseoli*. A. Infected leaflets; left, lower surface; right, upper surface; × 5/6. B. Reddish-brown stem lesion resulting from invasion of the node through an infected petiole; × 8/9. C. Plant with upper half of shoot blighted as a result of a lesion girdling the stem near the middle; × ¼. Plant completely blighted except for the green basal portion of the stem; × ¼. E. Infected pods; × 8/9.

tablespoonsful per pot. Sterile oats were added to control pots. Five days after inoculation seed were planted in the inoculated soil at the rate of 10 seeds per pot. For post-emergence inoculations the plants

were thinned to 4 plants per pot. They were inoculated by digging into the soil at the base of each plant and placing a tablespoonful of the oat inoculum in contact with the roots and base of the stem. Inoculations with *Macrophomina phaseoli* were made also by spraying the plants with suspensions of sclerotia or conidia from macerated 2 per cent malt agar cultures or diseased material from the field. All plants inoculated after emergence were held in an inoculation chamber for 48 hours. To determine the relationship between age of plant and susceptibility, seed were planted on successive dates so that groups of plants in various stages of maturity were available for inoculation. In determining the effect of soil sterilization on incidence of disease 2 field soils, a Tifton sandy loam and a Cecil clay loam, were potted. Half of the soil of each type was autoclaved, the other half left untreated. The pots were then inoculated and planted.

In seed treatment studies the seed were treated with ethyl mercury chloride (2% Ceresan) at the rate of 2 oz. per bushel. In field studies plots of 10 rows, each 16½ ft. long, were laid out. One pair of rows was uninoculated, sterile oats were added to 1 pair, and 1 pair each was inoculated with oat cultures of *Macrophomina phaseoli*, *Rhizoctonia solani*, and *Sclerotium rolfsii* at the rate of 1 pt. of inoculum per row. Seed were planted at the rate of 100 seeds per row. Treated seed were planted in 1 row of each pair, untreated seed in the other. The plots were replicated 5 times at each location. In all pre-emergence inoculations results were noted after 2 weeks; in all post-emergence inoculations, after 1 month.

EXPERIMENTAL WORK AND RESULTS.—*Fungi isolated.*—Isolations from the hypocotyls of snap bean plants with symptoms of damping off, stem canker, or blight in the vicinities of Tifton, Enigma, Quitman, and Pelham in South Georgia yielded the following fungi: *Macrophomina phaseoli*, *Rhizoctonia solani*, *Sclerotium rolfsii*, *Fusarium* spp., *Diaporthe phaseolorum* var. *sojae* (Lehman) Wehmeyer, *Neocosmopara vasinfecta* E. F. Sm., *Chaetomium* sp., and *Thielavia basicola* (Berk. & Br.) Zopf. Preliminary pathogenicity tests were made with 4 isolates of *M. phaseoli*, 4 of *R. solani*, 2 of *S. rolfsii*, 7 of *Fusarium* spp., 2 of *D. phaseolorum* var. *sojae*, 1 of *N. vasinfecta*, 1 of *Chaetomium* sp., and 2 of *T. basicola*. The percentage of noninfected plants that emerged from soil inoculated with *M. phaseoli*, *R. solani*, and *S. rolfsii* varied from 0-10. In pots inoculated with all other fungi the percentage emergence varied from 70-100, the same range found in control pots to which sterile oats had been added. Further studies were made with selected isolates of *M. phaseoli*, *R. solani*, and *S. rolfsii* since only these 3 fungi showed any indication of pathogenicity on snap bean.

The isolate of *M. phaseoli* used in most of these studies was the pycnidial strain No. 75 which has been described previously (8). Use of the term "pycnidial strain" refers merely to the ability of this strain to produce pycnidia in culture on agar media. All other isolates of this fungus from snap bean, whether from infected tissue or from conidia, produced only the sclerotial stage (*Sclerotium bataticola* Taub. or *Rhizoctonia bataticola* (Taub.) Butl.) on agar media. However, all of these isolates could be induced to form pycnidia in culture on sterilized snap bean stems (8), and pycnidia were produced on snap bean plants inoculated with sclerotia from a sclerotial strain. Crall (3) has pointed out that the pycnidial strain (No. 75) is unusual in that the pycnidia lack ostioles and no true sclerotia are produced in culture. All sclerotia-like bodies in the cultures seem to be capable of developing into pycnidia. Pycnidia produced in culture and on snap bean plants inoculated with this strain did not form ostioles but released the conidia through the rupture of the apex of the pycnidium. In the lack of sclerotia this strain resembles a pycnidial strain reported by Haigh (4). Haigh obtained an isolate from *Cajanus indicus* which on sub-culturing produced strains forming only sclerotia, strains forming both sclerotia and pycnidia, and one forming only pycnidia. The lack of an ostiole raises a serious question as to whether this strain should be included in *M. phaseoli*. However, in conidial characteristics it is similar to other strains of this species, and it produced typical symptoms of charcoal rot and ashy stem blight on inoculated snap bean plants. For the present, it is considered a morphologically aberrant strain of *M. phaseoli*.

*Relationship Between Age of Plant and Susceptibility.*—When introduced into autoclaved soil prior to planting, *Macrophomina phaseoli*, *Rhizoctonia solani*, and *Sclerotium rolfsii* severely infected snap bean seedlings (Table 3, Seed untreated). All caused considerable pre-emergence as well as post-emergence damping-off. Although death was often delayed until after the seedling stage, infection with all 3 fungi occurred chiefly during, or soon after, emergence. With *M. phaseoli* almost all infection occurred through the cotyledons during emergence. Occasionally plants recovered from slight early infections with *M. phaseoli* and *R. solani*.

Since infection with all 3 of these fungi appeared to take place during emergence or in the early seedling stage, experiments were conducted to determine the effect of age of bean plants on their susceptibility. Inoculations through the soil were made on 4 groups of plants ranging in age from 10-day-old seedlings to mature plants with dried pods. In addition, inoculations with *Macrophomina phaseoli* were made by spraying a suspension of conidia produced in culture by the pycnidial strain (No. 75) on the shoots. Results of these experiments are given in Table 1. A root rot caused by *Pythium* sp. was a source of some confusion in these and other greenhouse experiments. These results show that under conditions of high relative humidity *Sclerotium rolfsii* may cause severe infection of bean plants at any age. Plants inoculated with this fungus were wilted and dying when removed from the inoculation chamber 48 hours after inoculation. With

TABLE 1.—*Effect of age of snap bean plants on their susceptibility to blight fungi as determined from greenhouse inoculations in a potted, autoclaved compost-soil mixture*

| Inoculum | Seedling plants | | | Blooming plants | | | Plants with green pods | | | Plants with dried pods | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Inoculated | Infected | Dead | Inoculated | Infected | Dead | Inoculated | Infected | Dead | Inoculated | Infected | Dead |
| | Number | Per cent | Per cent | Number | Per cent | Per cent | Number | Per cent | Per cent | Number | Per cent | Per cent |
| None | 38 | 0 | 0 | 40 | 0 | 0 | 40 | 0 | 10[a] | 39 | 0 | 8[a] |
| *Macrophomina phaseoli* No. 75[c] | 46 | 100 | 7[a] | 40 | 100 | 8 | 39 | 100 | 26[b] | 39 | 100 | 15 |
| *Macrophomina phaseoli* No. 75[d] | 46 | 0 | 0 | 40 | 0 | 0 | 40 | 0 | 8[a] | 40 | 0 | 0 |
| *Rhizoctonia solani*[d] | 32 | 34 | 34 | 30 | 7 | 7 | 32 | 0 | 3[a] | 32 | 0 | 6[a] |
| *Sclerotium rolfsii*[d] | 32 | 97 | 97 | 32 | 97 | 97 | 32 | 100 | 100 | 32 | 100 | 100 |

[a]Apparently killed by *Pythium* sp. no evidence that death was caused by fungi used in making inoculations.
[b]Only 10 per cent definitely killed by *M. phaseoli*. *Pythium* sp. apparently responsible for death of 16 per cent of these plants.
[c]Conidia on shoots.
[d]Oat culture in soil.

*Rhizoctonia solani*, infection was heavy in the early seedling stage but decreased by the time the blooming stage was reached. Mature plants appeared to be nearly immune. When plants were inoculated through the soil with *M. phaseoli*, no infection was obtained at any stage after emergence. In contrast, when the shoots were inoculated with conidia of the same fungus, 100 per cent infection resulted at all stages of growth, the infections becoming increasingly severe with increasing age of the plants.

The pathogenicity of *Macrophomina phaseoli* was tested further on plants weakened by defoliation. Mature plants in pots were divided into 2 groups, and 1 group was completely defoliated. Plants in each group were then inoculated through the soil with oat cultures of a non-pycnidial strain and on the shoots with a suspension of sclerotia from a culture of the same non-pycnidial strain, a suspension of conidia from agar cultures of the pycnidial strain (No. 75), and a suspension of conidia from bean stems infected naturally in the field. Results (Table 2) show the weakening effect of defoliation in that more than ¼ of the control plants died during the subsequent month. This was reflected in the severe infection produced on defoliated plants when the shoots were inoculated with either conidia or sclerotia. On the other hand, no infection resulted from inoculations with sclerotia and mycelium through the soil even on plants weakened by defoliation.

*Seed Treatment.*—The fact that both *Rhizoctonia solani* and *Sclerotium rolfsii* could cause post-emergence infection indicated that seed treatment might not effectively control the diseases they cause. On the other hand, the fact that infection with *Macrophomina phaseoli* occurred almost exclusively through the cotyledons during emergence offered hope of controlling charcoal rot by seed treatment. Experiments were designed, therefore, to determine the effectiveness of seed treatment in controlling these bean diseases. In greenhouse tests (Table 3) such treatment increased emergence in pots inoculated with *R. solani* and *S. rolfsii* but had little beneficial effect on stand at maturity. In pots inoculated with *M. phaseoli* excellent control was obtained from seed treatment.

Control of charcoal rot by seed treatment in the greenhouse led to field trials of this method. Replicated plot tests were planted on 2 dates at Tifton and at Experiment in 1946. In 1947 only the tests at Tifton were made. At Tifton the early plantings were made about the first of April, the late plantings

TABLE 2.—*Effect of defoliation on the susceptibility of snap bean plants to Macrophomina phaseoli*

| Inoculum | Normal | | | Defoliated | | |
|---|---|---|---|---|---|---|
| | Inoculated | Infected | Dead | Inoculated | Infected | Dead |
| | Number | Per cent | Per cent | Number | Per cent | Per cent |
| None—sterile oats in soil | 12 | 0 | 0 | 11 | 0 | 27 |
| Oat culture in soil (non-pycnidial strain) | 12 | 0 | 0 | 11 | 0 | 9 |
| Sclerotia from culture on shoot (non-pycnidial strain) | 12 | 83 | 58 | 12 | 100 | 75 |
| Conidia from culture on shoot (pycnidial strain No. 75) | 12 | 100 | 33 | 12 | 100 | 92 |
| Conidia from field on shoot | 12 | 100 | 75 | 12 | 100 | 100 |

TABLE 3.—*Effect of seed treatment with "2% Ceresan"[a] on infection of snap bean plants by blight fungi as determined from greenhouse inoculations in a potted, autoclaved compost-soil mixture*

| Inoculum | Seed untreated | | | | | Seed treated | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Seed | Total emerged | Non-infected emerged | Total mature | Non-infected mature | Seed | Total emerged | Non-infected emerged | Total mature | Non-infected mature |
| | Number | Per cent | Per cent | Per cent | Per cent | Number | Per cent | Per cent | Per cent | Per cent |
| None—sterile oats | 80 | 93 | 93 | 93 | 93 | 80 | 98 | 98 | 98 | 98 |
| *Macrophomina phaseoli* | 80 | 64 | 26 | 35 | 29 | 80 | 91 | 88 | 79 | 76 |
| *Rhizoctonia solani* | 80 | 43 | 13 | 20 | 14 | 80 | 66 | 26 | 33 | 16 |
| *Sclerotium rolfsii* | 80 | 26 | 19 | 14 | 10 | 80 | 41 | 33 | 26 | 14 |

[a]Ethyl mercury chloride.

about the middle of May At Experiment the early planting was made at the end of April, the late planting about the middle of June. Results (Table 4) were quite different from those obtained in the greenhouse. Seed treatment increased stands in rows inoculated with *Rhizoctonia solani* and *Sclerotium rolfsii*, but the stands resulting even from treated seed were so poor that these increases were of little practical significance. In rows inoculated with *Macrophomina phaseoli* seed treatment produced only slight increases in stands. Although the seed were planted in contact with the inoculum, there was little charcoal rot. There was a considerable amount of infection of the cotyledons on seedlings from untreated seed as compared with none on those from treated seed, but the diseased cotyledons soon dropped off and there was little infection of the stems. Seed treatment had little effect in noninoculated soil. The lower percentages of emergence in the late plantings may be explained by the fact that, while in the early plantings *R. solani* caused infection only in rows into which it had been introduced, it was generally distributed in the same fields at the time the late plantings were made and caused severe infection in all rows.

*Effect of Soil Sterilization on Incidence of Disease.*—In an attempt to explain the disparity in results obtained in the greenhouse and in the field, inoculations were made in the greenhouse in pots of autoclaved and nonsterilized field soil from both Tifton and Experiment. Treated and untreated seed were planted in both autoclaved and nonsterilized lots of soil of each type. Results obtained with autoclaved field soils (Table 5) paralleled those obtained previously in greenhouse inoculations with an autoclaved compost-soil mixture (Table 3) except that emergence was generally poorer in these heavier soils. Results with nonsterilized field soils (Table 5) were similar to those obtained in the field (Table 4). *Macrophomina phaseoli* caused little infection of beans in nonsterilized soil but produced a high percentage of infection in autoclaved soil. When infection occurred, it was almost completely controlled by seed treatment. Although *Rhizoctonia solani* was more virulent in autoclaved soil, it also produced severe infection in nonsterilized soil. This fungus was controlled to some extent by seed treatment. *Sclerotium rolfsii* infected nearly 100 per cent of the plants in both autoclaved and nonsterilized soil and was not controlled by seed treatment. The addition of sterile oats to nonsterilized soil greatly decreased the percentage of seedlings that emerged, this added organic matter doubtless favoring the development of pathogens normally present in these field soils.

TABLE 4.—*Effect of seed treatment with "2% Ceresan"[a] on infection of snap bean plants by blight fungi in the field in normal and artificially inoculated soils*

| Inoculum | Percentage of non-infected seedlings emerged | | | |
|---|---|---|---|---|
| | Early plantings | | Late Plantings | |
| | Seed untreated | Seed treated | Seed untreated | Seed treated |
| None—control | 69[b] | 73 | 51 | 56 |
| None—sterile oats | 60 | 69 | 41 | 42 |
| *Macrophomina phaseoli* | 55 | 67 | 34 | 45 |
| *Rhizoctonia solani* | 13 | 35 | 13 | 24 |
| *Sclerotium rolfsii* | 2 | 27 | 1 | 4 |

[a]Ethyl mercury chloride.
[b]Each percentage derived from mean of 15 replications.

DISCUSSION.—Results of inoculations with *Macrophomina phaseoli* illustrate the difference in behavior of a single fungus when the inoculum is air borne from plant to plant and when it develops in the soil. They show that air borne conidia may cause shoot infection of bean plants at any stage of development provided a high relative humidity is maintained for a period sufficient to permit germination and penetration. Apparently on the more selective substrate furnished by living stems and leaves the fungus may spread from plant to plant without being subjected to serious competition with other organisms. On the other hand, data presented in Tables 3, 4, and 5 indicate that, when *M. phaseoli* is inoculated into nonsterilized field soil, its potential virulence, as demonstrated by inoculations in autoclaved soil, is greatly reduced, presumably as a result of competition with other soil organisms. The failure of *M. phaseoli* to attack bean seedlings even when large amounts of inoculum were

TABLE 5.—*Effect of seed treatment with "2% Ceresan"[a] on infection of snap bean plants by blight fungi in greenhouse inoculations in potted normal and autoclaved soils*

| Inoculum | Seed untreated | | | Seed treated | | |
|---|---|---|---|---|---|---|
| | Seed | Total emerged | Non-infected emerged | Seed | Total emerged | Non-infected emerged |
| | Number | Per cent | Per cent | Number | Per cent | Per cent |
| Tifton sandy loam (nonsterilized) | | | | | | |
| None—control | 30 | 77 | 77 | 30 | 67 | 67 |
| None—sterile oats | 30 | 57 | 47 | 30 | 80 | 70 |
| *Macrophomina phaseoli* | 30 | 83 | 73 | 30 | 77 | 77 |
| *Rhizoctonia solani* | 30 | 50 | 30 | 30 | 57 | 43 |
| *Sclerotium rolfsii* | 30 | 3 | 3 | 30 | 0 | 0 |
| Tifton sandy loam (autoclaved) | | | | | | |
| None—control | 30 | 83 | 83 | 30 | 93 | 93 |
| None—sterile oats | 30 | 83 | 83 | 30 | 90 | 90 |
| *Macrophomina phaseoli* | 30 | 27 | 20 | 30 | 63 | 63 |
| *Rhizoctonia solani* | 30 | 27 | 23 | 30 | 47 | 37 |
| *Sclerotium rolfsii* | 30 | 3 | 0 | 30 | 10 | 7 |
| Cecil clay—loam (nonsterilized) | | | | | | |
| None—control | 30 | 60 | 57 | 30 | 90 | 90 |
| None—sterile oats | 30 | 33 | 27 | 30 | 40 | 40 |
| *Macrophomina phaseoli* | 30 | 53 | 50 | 30 | 83 | 80 |
| *Rhizoctonia solani* | 30 | 63 | 50 | 30 | 80 | 63 |
| *Sclerotium rolfsii* | 30 | 0 | 0 | 30 | 0 | 0 |
| Cecil clay—loam (autoclaved) | | | | | | |
| None—control | 30 | 70 | 70 | 30 | 83 | 83 |
| None—sterile oats | 30 | 77 | 73 | 30 | 87 | 87 |
| *Macrophomina phaseoli* | 30 | 33 | 7 | 30 | 60 | 60 |
| *Rhizoctonia solani* | 30 | 20 | 13 | 30 | 60 | 47 |
| *Sclerotium rolfsii* | 30 | 0 | 0 | 30 | 3 | 3 |

[a]Ethyl mercury chloride.

introduced into nonsterilized field soils is obscured to some extent in the data given in Table 5 by the deaths of plants resulting from infection with other pathogens, such as *Rhizoctonia solani* and *Sclerotium rolfsii*, normally present in these soils. A clearer demonstration might be obtained by making inoculations in soil previously sterilized to eliminate any pathogens present and reinoculated with specific antagonistic saprophytes. Variations in the development of charcoal rot on beans at different times and locations may be explained by differences in the nature of the soil community. Such an explanation is suggested by Cooper and Chilton (2) who indicate that root rot of sugar cane caused by *Pythium arrhenomanes* is less severe in soils in which the antibiotic potential of soil Actinomycetes is highest. It might be expected that various environmental factors would greatly influence competitions among microorganisms in the soil and, consequently, the soil population and virulence of *M. phaseoli*. Under conditions of extremely high soil temperatures, for example, *M. phaseoli* might become dominant and cause severe infection. Kendrick (7) found that in California charcoal rot in beans was most severe during periods of high temperatures. However, conflicting data on cowpea (9) and lima bean (1) suggest that the primary effect of temperature is on the development of the host. Data on the effect of temperature on *M. phaseoli* in mixed communities in the soil are not available.

The failure of *Macrophomina phaseoli* to infect snap bean plants when post-emergence inoculations were made through the soil is considered to be the result of increased resistance of older plants. However, an alternative explanation should be considered. It is possible that, as in Pythium root rot of sugar cane (6), the lack of infection may have resulted from the inhibition of the fungus by other microorganisms which had recontaminated the soil during the intervals between planting and inoculation.

Field observations on *Rhizoctonia solani* indicate that environmental factors may influence soil borne diseases at 2 stages: 1) in the development of inoculum, and 2) during the course of infection. In late plantings of beans at Tifton, *R. solani* was generally distributed throughout the field and caused severe infection in all rows. Early plantings in the same field were not infected with *R. solani* except in rows into which it had been introduced. Apparently at the time the early plantings were made environmental factors favored the growth of the fungus sufficiently to permit infection, but these conditions had not been operative long enough to permit the development of a soil population to furnish inoculum.

Results on the effect of seed treatment were not conclusive enough to justify the recommendation of seed treatment as a control measure for snap bean diseases in Georgia. They suggest, however, that in localities in which charcoal rot is a problem seed treatment

would at least be worthy of field trials as a control measure. On the other hand, observations indicate that these diseases may be avoided by early planting.

SUMMARY

Greenhouse and field studies have shown that the fungi chiefly responsible for blights of snap bean in Georgia are *Macrophomina phaseoli*, *Rhizoctonia solani*, and *Sclerotium rolfsii*.

*Macrophomina phaseoli* causes charcoal rot, a seedling disease in which infection by mycelium in the soil occurs primarily through the cotyledons during emergence, and ashy stem blight and leaf spot, a disease of older plants in which infection is accomplished largely by air-borne conidia. A high percentage of infection could be obtained at any time by inoculating the shoots with conidia, the infection becoming more severe with increasing age of the plants. No infection resulted from soil inoculations made after emergence. Charcoal rot was severe in autoclaved soil inoculated prior to planting and could be completely controlled by seed treatment with "2% Ceresan." Little infection resulted from inoculations with *M. phaseoli* in unsterilized field soils.

*Rhizoctonia solani* and *Sclerotium rolfsii* caused infection in nonsterilized soil as well as in autoclaved soil. Beans were highly susceptible to *S. rolfsii* at all ages but became increasingly resistant to *R. solani* with increasing age.

Seed treatment failed to give practical control of these diseases in field trials. Field observations indicate that they may be avoided by early planting.

GEORGIA EXPERIMENT STATION
EXPERIMENT, GEORGIA

LITERATURE CITED

1. ANDRUS, C. F. 1938. Seed transmission of Macrophomina phaseoli. Phytopathology **28**: 620-634.
2. COOPER, W. E., AND S. J. P. CHILTON. 1950. Studies on antibiotic soil organisms. I. Antinomycetes antibiotic to Pythium arrhenomanes in sugar-cane soils of Louisiana. Phytopathology **40**: 544-552.
3. CRALL, J. M. 1948. Charcoal rot of soybean caused by Macrophomina phaseoli (Maubl.) Ashby. Thesis. University of Missouri.
4. HAIGH, J. C. 1930. Macrophomina phaseoli (Maubl.) Ashby and Rhizoctonia bataticola (Taub.) Butler. Ann. Ceylon Roy. Bot. Gard., Peradeniya **11**: 213-249.
5. HARTER, L. L., AND W. J. ZAUMEYER. 1944. A monographic study of bean diseases and methods for their control. U. S. Dept. Agr. Tech. Bull. No. 868: 1-160.
6. JOHNSON, L. F. 1952. Recontamination of sterilized soil with relation to Pythium root rot of sugar cane and corn. (Abs.) Phytopathology **42**: 12-13.
7. KENDRICK, J. B. 1933. Seedling stem blight of field beans caused by Rhizoctonia bataticola at high temperatures. Phytopathology **23**: 949-963.
8. LUTTRELL, E. S. 1946. A pycnidial strain of Macrophomina phaseoli. Phytopathology **36**: 978-980.
9. TOMPKINS, C. M., AND M. W. GARDNER. 1935. Relation of temperature to infection of bean and cowpea seedlings by Rhizoctonia bataticola. Hilgardia **9**: 219-230.
10. WEIMER, J. L., AND E. S. LUTTRELL. 1945. A canker of cowpea and Macrophomina leaf spot of cowpea and snap bean. U. S. Dept. Agr. Plant Dis. Reptr. **29**: 127-129.

## ANTIBIOTICS AND ANTAGONISTIC MICROORGANISMS AS CONTROL AGENTS AGAINST DAMPING-OFF OF ALFALFA[1]

K. F. Gregory, O. N. Allen, A. J. Riker, and W. H. Peterson

Damping-off and root rot are major economic diseases of alfalfa commonly caused by species of *Pythium* (2, 4, 5, 11, 15, 21). These diseases of alfalfa result in reduced crop stands in the field but are less commonly recognized there than in the greenhouse (10, 14, 21).

The use of microbial anatagonists has given some promise of controlling damping-off. *Trichoderma lignorum* (Tode) Harz, added to a sterilized acid soil, was found to reduce damping-off of citrus seedlings caused by *Rhizoctonia solani* Kühn (26). When this antagonist was grown on sterilized whole oats and added to infested soil, seed decay and damping-off of cucumbers and peas were decreased (1, 26). Various isolates of streptomycetes have caused a reduction of root rot in young sugar cane and corn plants infested with species of *Pythium* (6, 24).

Reports on the use of antibiotics for control of damping-off have been sparse. According to Meredith and Semeniuk (16), corn plants grown with *Pythium graminicola* Subr. in soil treated with an impure streptomycete antibiotic were taller and had longer roots than those grown in nontreated soil containing *P. graminicola* only. In a study made by Pine (18) clavacin, gliotoxin, glutinosin and tyrothricin were especially active against *P. debaryanum*. Clavacin and glutinosin were considerably more potent against *P. debaryanum* than against *Rhizobium meliloti*, gliotoxin showed approximately equal activity against both organisms, whereas tyrothricin was more toxic to the rhizobia than to the fungus. All these antibiotics were toxic to alfalfa seedlings and less effective as control agents than was 1 per cent ethyl mercury phosphate. Appreciable control of damping-off was obtained by pelleting alfalfa seeds in peat moss treated with the metabolic solutions, with and without spores, of *Penicillium patulum* Bain. (forming clavacin) or *T. lignorum* (forming gliotoxin). Less effective control was obtained when *Metarrhizum glutinosum* S. Pope (forming glutinosin) was used.

[1] Accepted for publication July 28, 1952.
This work was supported by a grant from The Albert Dickinson Company, Chicago, Illinois. Published with the approval of the Director of the Wisconsin Agricultural Experiment Station.
The authors are indebted to Eugene Herrling for assistance with the illustrations.

TABLE 1.—*Identity and source of the test organisms*

| Organism | Strain Number | Source |
|---|---|---|
| Antagonists and antibiotic-producing strains: | | |
| *Penicillium patulum* Bain. | NRRL 1952 | Northern Regional Research Laboratory, Peoria, Illinois |
| *Trichoderma lignorum* (Tode) Harz | NRRL 1828 | . . . do . . . |
| *Streptomyces* sp. | A66 | Isolated from soil, Madison, Wisconsin |
| . . . do . . . | A67 | . . . do . . . |
| *Bacillus* sp. | B6 | . . . do . . . |
| Causal agents of damping-off: | | |
| *Pythium debaryanum* Hesse | 285 | U.S.D.A., Beltsville, Maryland |
| . . . do . . . | P, C-31 | Culture collection, Dept. of Plant Pathology, Univ. Wisconsin |
| *Pythium graminicola* Subr. | 11 | Iowa State College, Ames, Iowa |
| . . . do . . . | 53 | . . . do . . . |
| *Pythium irregulare* Buis. | 246 | Culture collection, Dept. of Plant Pathology, Univ. Wisconsin |
| . . . do . . . | PS, G-11 | . . . do . . . |
| *Pythium ultimum* Trow | P, C-18 | . . . do . . . |
| *P. vexans* D'By. | Mi, C-18 | . . . do . . . |

Involved in this over-all problem is the question of compatibility between antagonists and their by-products with rhizobia, since much of the leguminous seed planted today is treated with these symbiotic nitrogen-fixing bacteria before planting. As stated by Ruhloff and Burton (20), sometimes seed inoculation with rhizobia and at other times treatment with protectant chemicals might be preferred. Rapid methods of predetermining the need for either of these treatments are lacking.

*In vitro* studies using antibiotics and microbial antagonists against certain damping-off fungi are described by the authors in an earlier paper (12). The present paper contains data from greenhouse tests in which these agents were used against *Pythium* species causing damping-off of alfalfa seedlings.

MATERIALS[2] AND METHODS.—The organisms used in this study are listed in Table 1.

Plainfield sand (pH 5.6 to 6.2) taken from a forest floor near Wisconsin Rapids, Wisconsin, was used in all experiments except as noted. In its natural condition this soil was normally free from damping-off fungi. Throughout the tests the soil was maintained at nearly 90 per cent of its moisture-holding capacity by placing the 4-in. pots in large pans containing a ¼-in. layer of water (19). A transparent Flexiglass frame was placed over the pans to maintain the desired humidity and also to prevent spread of infection.

Approximately 65 alfalfa seeds of either the Kansas or Dakota variety were planted in each pot. The many samples of alfalfa seed required were obtained by matching the weights of the seed lots with that of a counted sample. Variations in number of seeds among the various batches were insignificant.

Each pot inoculated with the damping-off fungi received 5 discs (dia. 18 mm.) of a 5-day growth of the fungus on a potato glucose agar plate. In 2 experiments the species of *Pythium* were cultivated on sterile oat hulls, which were then thoroughly mixed with the soil. One ml. of a 4-day-old yeast-water mannitol broth culture of *Rhizobium meliloti* 107, an effective nitrogen-fixing strain, was added to the seeds in each pot at the time of planting.

All damped-off seedlings were replaced by toothpicks which differed in color according to the time of observations. In this way data concerning the extent and progressive rate of damping-off were readily compiled from each series.

Toxicity of the antibiotics for the alfalfa seeds was determined in 2 ways. In one procedure various lots of seeds were soaked 4 hours in dilutions of a graded series of the antibiotic, then were removed from the solutions, dried overnight at 37° C., and planted in Plainfield sand in the greenhouse. Viability of the treated seeds was compared with that of seeds soaked in water only. In the other method the seeds were planted in pots of Plainfield sand which had been moistened with various concentrations of the antibiotics. The numbers of plants surviving and their dry weights were used as indices of phytotoxicity.

In view of the results reported by Pine (18), pelleting of alfalfa seeds was considered a promising means of establishing the antibiotic-producing organisms in the rhizosphere. Two pelleting mixtures were used: one consisted of 60 per cent brown peat, 25 per cent methyl cellulose and 15 per cent glucose, and the other comprised 75 per cent brown peat and 25 per cent methyl cellulose. The components of each mixture were blended together and moistened with a broth culture of the antagonist to form a paste. After the seeds had been homogeneously stirred into this material, the mixture was forced through a pastry tube equipped with a circular nozzle. (dia. 3 mm.). Subsequent to partial drying, the ribbons were cut into cylindrical pellets averaging 3-4 mm. long and about 2 seeds each.

Each experiment comprised 3 replications of each treatment and was repeated 2 or more times. The positions of the pots on the greenhouse benches were randomized in each experiment.

[2] The antibiotics, actidione and fradicin, were generously supplied by the Upjohn Company, Kalamazoo, Michigan, and the Commercial Solvents Corporation, Terre Haute, Indiana, respectively.

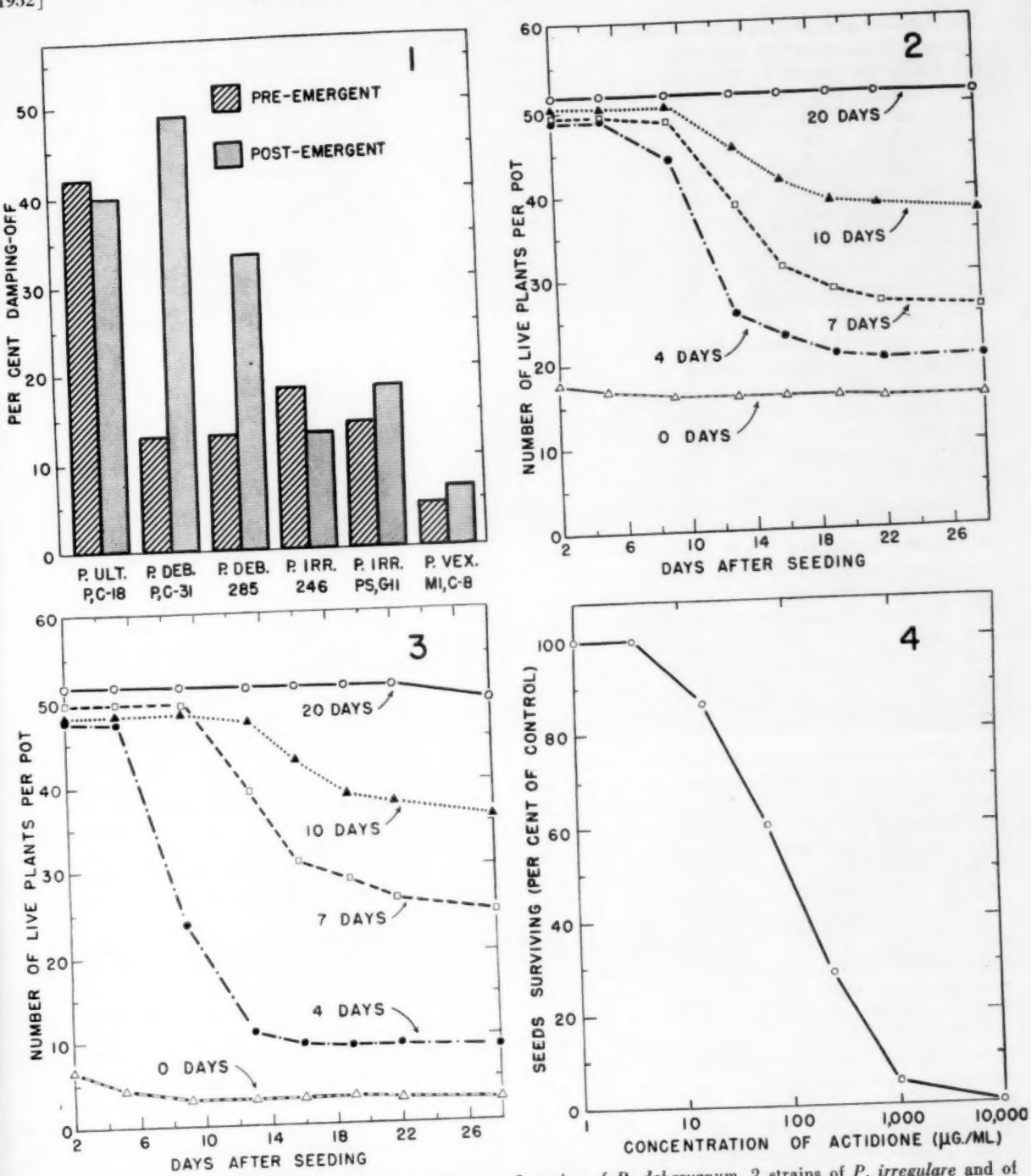



FIG. 1–4.—FIG. 1. Pathogenicity of *Pythium ultimum*, 2 strains of *P. debaryanum*, 2 strains of *P. irregulare* and of *P. vexans* for alfalfa seedlings.—FIG. 2. Survival of alfalfa seedlings following inoculation with *P. debaryanum* 285 at 0, 4, 7, 10 and 20 days after seeding.—FIG. 3. Survival of alfalfa seedlings following inoculation with *P. ultimum* P, C-18 at 0, 4, 7, 10 and 20 days after seeding.—FIG. 4. Percentage of alfalfa seeds surviving 4 hours immersion in actidione solutions.

RESULTS.—The pathogenicity for alfalfa of the species of *Pythium* listed in Table 1 was ascertained by greenhouse tests. The percentages of pre- and post-emergent damping-off caused by 6 strains of the 4 *Pythium* species appear in Figure 1. Marked variation in the severity of infection was shown by the different species; only minor variation occurred among the strains of a single species. *P. ultimum* induced the most severe infection and generally destroyed a majority of the plants before they emerged from the soil.

The 2 strains of *P. debaryanum* caused extensive post-emergent damping-off. A relatively low incidence of the disease was caused by the strains of *P. irregulare* and *P. vexans*. The strains of *P. graminicola* were not pathogenic for alfalfa and therefore are not included in Figure 1.

Severity of damping-off was affected by temperature. A greater percentage of plants survived infection when the maximum daily temperatures varied between 85°-90° F. than between 70°-75° F. Regardless of the temperature, *P. ultimum* was always more pathogenic than was *P. debaryanum*.

The approximate age at which alfalfa seedlings developed resistance to infection by the *Pythium* spp. was ascertained by inoculating seeded pots of Plainfield sand at successive time intervals with *P. debaryanum* 285 (Fig. 2) and *P. ultimum* (Fig. 3). A small percentage of plants survived infection even when these infective agents were present at the time the seeds were planted. In general, the older the seedlings at time of inoculation, the greater their resistance to attack by the *Pythium* spp. Only about 30 per cent of the 2-day-old seedlings inoculated with *P. debaryanum* survived; more than 75 per cent survived when the plants were 10 days old at the time of inoculation. Little or no loss of plants occurred when the inoculations were made 14 or 20 days after seeding. The results indicated that the biological treatments must be effective throughout 2-3 weeks after seeding in order to be of value in protecting seedlings from damping-off by *Pythium* spp.

*Results with pre-formed antibiotics.* — Laboratory tests have shown that actidione, fradicin and the antibiotics from *Streptomyces* sp. A67 and *Bacillus* sp. B6 inhibited species of *Pythium* without harming rhizobia (12). Of these, only actidione and fradicin persisted in the soil for the 14-day period when alfalfa seedlings are most susceptible to damping-off.

a) *Actidione.* The numbers of seeds remaining viable after treatment for 4 hours in various concentrations of actidione are presented graphically in Figure 4. As was shown previously (12), the minimum concentrations of actidione required in agar dilution assays for the complete inhibition of *P. debaryanum* 285 and *P. ultimum* P, C-18 were 14.5 μg./ml. and 23 μg./ml., respectively. As is shown here, this antibiotic is harmful to alfalfa seeds at concentrations lower than those required to inhibit these fungi completely.

The phytotoxicity of actidione was further emphasized by tests in which alfalfa seeds were planted in pots of soil treated with 5 concentrations of actidione (Fig. 5a and b). Seedlings were slightly retarded in growth when only 0.4 μg. of actidione was present in 1 ml. of soil solution and were severely stunted by greater concentrations. Nodulation was not noticeably affected by 6.2 μg. of actidione per ml. of soil solution but was markedly inhibited by greater amounts. Presumably this reduction in number of nodules resulted from the reduced vigor of the seedlings, since concentrations of actidione as great as 10 mg./ml. did not inhibit rhizobia in laboratory tests (12).

TABLE 2.—*Effect of actidione added to Plainfield sand on the control of damping-off of alfalfa*

| Concentration of actidione | Plants surviving 23 days | | |
|---|---|---|---|
| | Without *Pythium* sp. | *P. debaryanum* 285 | *P. ultimum* P, C-18 |
| μg./ml. | Per cent | Per cent | Per cent |
| 0.0 | 100 | 19 | 6 |
| 0.4 | 95 | 32 | 14 |
| 1.6 | 91 | 36 | 12 |
| 6.2 | 80[a] | 44[b] | 50[a] |
| 25 | 43[a] | 60[a] | 54[a] |
| 100 | 4[a] | 8 | 6 |

[a]Significant difference at the 1 per cent level from the series with no actidione.
[b]Significant difference at the 5 per cent level from the series with no actidione.

Damping-off of alfalfa was prevented completely by solutions containing 25 μg./ml. of actidione added to Plainfield sand which was inoculated with *P. debaryanum* 285 and *P. ultimum* (Table 2). Although the numbers of seedlings surviving in pots inoculated with *P. debaryanum* and *P. ultimum* were increased 3- and 9-fold, respectively, as a result of this antibiotic treatment, seedlings were badly stunted (Fig. 6). Concentrations of actidione less than 25 μg./ml. only partially protected the seedlings from fungus attack; 100 μg./ml. of actidione killed more plants than did the pathogens.

These results are in general agreement with those obtained by others (8, 25). Despite the importance of this antibiotic as a fungistatic agent in media (17, 25), against powdery mildews (7, 25) and in the control of covered smut of wheat (13), its use on certain plants is limited by its phytotoxicity. In these studies the injurious effect of actidione on alfalfa seedlings was sufficiently severe to preclude its use as a protectant against damping-off fungi.

b) *Fradicin.*—Determination of the phytotoxicity of fradicin was complicated by its low solubility in water. Accordingly, a stock solution containing 400 μg. of fradicin per ml. of absolute methyl alcohol was prepared prior to making dilutions with distilled water. The largest amount of fradicin employed, a 1:10 dilution, comprised, therefore, 40 μg./ml. in 10 per cent methyl alcohol. Treatment of alfalfa seeds for 4 hours in this solution caused a 56 per cent reduction of viable seed, but an equivalent decrease was also produced by 10 per cent methyl alcohol alone. No reduction in the number of viable seeds was caused by solutions containing 10 μg./ml. and less of fradicin.

Fradicin was used also in greenhouse tests to treat the soil and alfalfa seeds planted in both naturally infested Carlisle muck and in Plainfield sand inoculated with *P. debaryanum* (Table 3). In one series 2 solutions containing 9 μg./ml. and 34 μg./ml. of fradicin, respectively, were mixed with the air-dried soils. In another, 1 part of fradicin powder was mixed with 9 parts of powdered talc and used as a dust on the seeds.

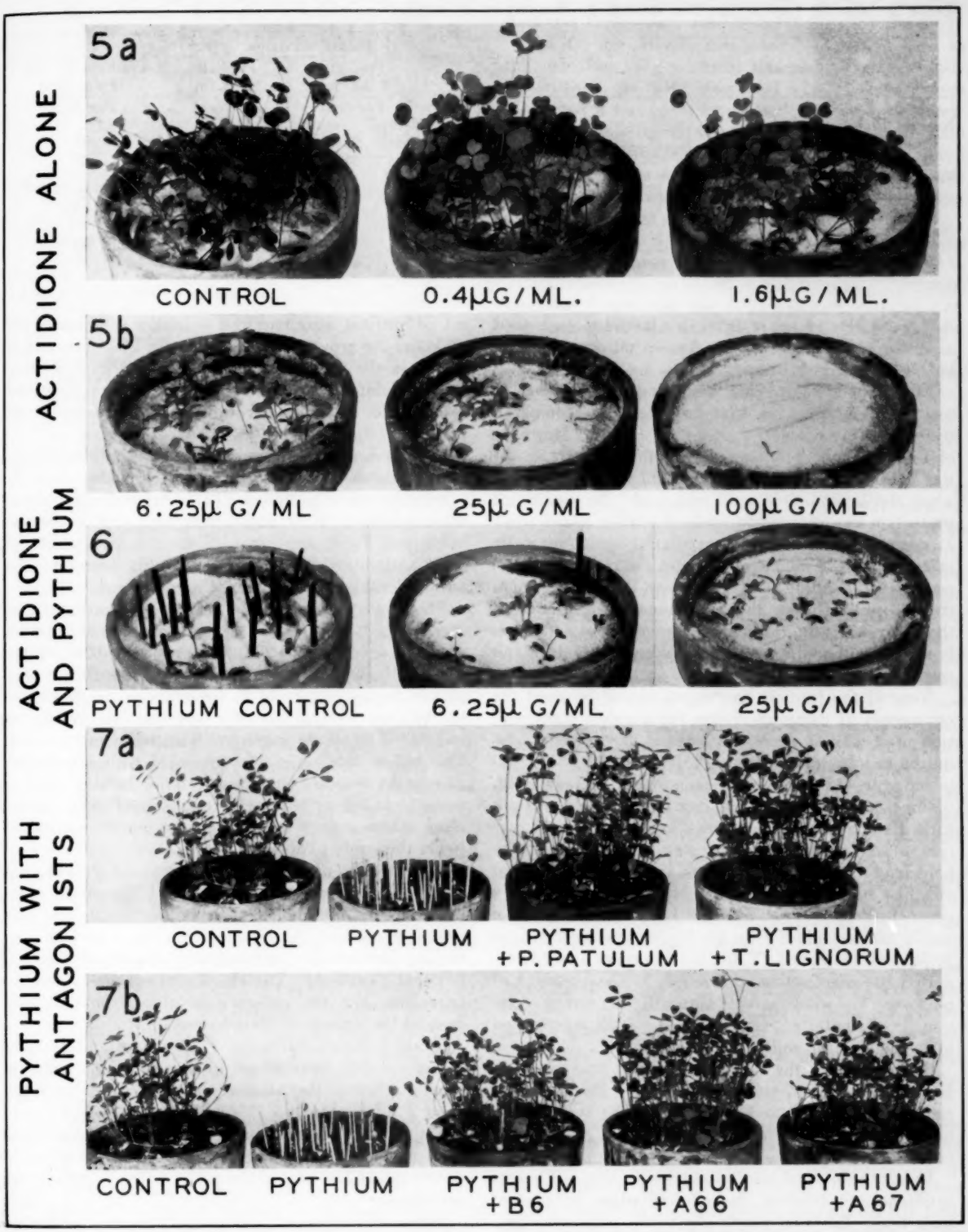



FIG. 5–7.—FIG. 5a and b. Toxicity of various amounts of actidione per ml. to alfalfa seedlings. White sand was added to the surface of the soil as a photographic aid.—FIG. 6. Effect of amounts of actidione on alfalfa inoculated with *P. debaryanum* 285. Each toothpick indicates one damped-off seedling.—FIG. 7a and b. Control of damping-off in natural Plainfield sand by mass inoculation of the soil with cultures antagonistic to *P. debaryanum* (summer experiment).

TABLE 3.—*Use of fradicin for the control of damping-off of alfalfa*

| Treatment | Plants surviving 29 days | | | |
|---|---|---|---|---|
| | Control | Fradicin 9 μg./ml. | Fradicin 34 μg./ml. | Fradicin in talc 10 μg./seed |
| | | Per cent | Per cent | Per cent |
| Natural Plainfield sand | | | | |
| Control | 100 | 93 | 62 | 55 |
| *P. debaryanum* added | 34 | 34 | 39 | 18 |
| Sterile Plainfield sand | | | | |
| Control | 94 | 62 | 29 | 86 |
| *P. debaryanum* added | 28 | 31 | 25 | 20 |
| Naturally infested Carlisle muck | | | | |
| Control | 44 | 39 | 43 | 48 |
| *P. debaryanum* added | 33 | 44 | 39 | 37 |

Approximately 10 μg. of fradicin adhered to each seed as determined by weighing a known number of seeds before and after dusting.

All fradicin treatments decreased the number of seedlings emerging in Plainfield sand as compared with those in the control series (Table 3). Mortality of the seedlings following the fradicin-talc treatment was greater in the natural Plainfield sand series than when sterile Plainfield sand was used. No significant protection was afforded by fradicin in these series when *P. debaryanum* was added. Similarly, in the naturally infested Carlisle muck fradicin did not reduce the percentage of seedling survival below that of the nontreated soil. Unlike the actidione-treated seedlings, those surviving the fradicin treatments were not greatly stunted although the roots were frequently dark and restricted in size.

None of the fradicin treatments of soil lessened the occurrence of damping-off, yet the greatest concentration used was 61 times the amount, 0.56 μg./ml., required to inhibit completely *P. debaryanum* 285 in nutrient agar (12). This aforementioned anomaly is explained undoubtedly by the fact that fradicin was adsorbed or inactivated. As was found earlier, 91 per cent of the fradicin present was adsorbed in Plainfield sand at pH 5.6 (12). Thus, in assuming here that only the unadsorbed antibiotic fraction was active, a concentration of only about 3.1 μg./ml. of fradicin would have remained from the highest concentration of 34 μg./ml. employed (Table 3). Swart et al. (23) reported the inactivation of fradicin in the presence of glucose. To what extent naturally occurring compounds of similar nature accounted for the inactivation of fradicin in the soil was not determined.

Nodulation by the rhizobia was not prevented by any of the fradicin treatments. Where the antibiotic caused a darkening and inhibition of the root system, however, the numbers of nodules were reduced accordingly.

*Results with antibiotic production in situ.*—Whereas actidione and fradicin showed limitations because of their toxic effects on young plants, the antibiotics produced by *Streptomyces* sp. A67 and *Bacillus* sp. B6 caused no harm to alfalfa seedlings at concentrations as high as 80 units per ml. One unit of antibiotic is defined here as the smallest amount of antibiotic per ml. of nutrient agar required to inhibit completely for 48 hours the growth of *P. debaryanum* 285. The plants grown in the presence of these latter antibiotics were well nodulated. However, these antibiotics appeared to be of limited value as control agents of damping-off because of their instabilities in soil.

Certain organisms are capable of producing antibiotic substances in nonsterile soil provided suitable conditions are maintained (12). Accordingly, it was reasoned that if these antagonists were added with the seeds and conditions provided for the production of their antibiotics *in situ*, the instability factor of the antibiotics might be minimized in importance.

Preliminary experiments using *T. lignorum* and *Streptomyces* sp. A67 as antagonists in combination with *P. debaryanum* 285 were conducted in sterile Miami silt loam at pH 6.2. In one series 10 ml. of a shaken liquid culture of *T. lignorum* or *Streptomyces* sp. A67 were centrifuged, the mycelium and spores resuspended in sterile water and used to inoculate 1 pot. The soil in this series was amended with 1 per cent glucose by weight. Other pots of soil lacking glucose were included as controls. In a second series these same cultures were mixed with the materials used to pellet the seeds (Table 4).

The severity of damping-off caused by *P. debaryanum* was much greater in the sterile Miami silt loam used in this experiment than in the nonsterile soils named elsewhere in this investigation. In soil containing glucose the incidence of damping-off was 100 per cent (Table 4). The absence of extraneous microorganisms and the presence of glucose undoubtedly favored the growth of *P. debaryanum*.

Table 4 shows that when *T. lignorum* and *Streptomyces* sp. A67 were added to this sterilized soil containing glucose the alfalfa seedlings were protected from *P. debaryanum*. *T. lignorum* incorporated into pellet mixtures both with and without glucose also gave considerable reduction of the disease. *Streptomyces* sp. A67 as part of the pellet failed to protect the seedlings.

More seedlings emerged in the presence of *T. lignorum* than in the control pots not containing *P. debaryanum* or an antagonist. This result was obtained in almost every experiment in which *T. lignorum* was added either to sterile or to nonsterile soil. Presum-

TABLE 4.—*Reduction of damping-off of alfalfa in sterile Miami silt loam by means of organisms antagonistic to Pythium debaryanum*

| Treatment | Seedlings surviving 14 days[a] | |
|---|---|---|
| | Without *Pythium debaryanum* | With *Pythium debaryanum* |
| | Per cent | Per cent |
| Antagonists added to soil | | |
| None (control) | 100 | 4 |
| Glucose | 93 | 0 |
| Glucose and *T. lignorum* | 110 | 112 |
| Glucose and *Streptomyces* sp. A67 | 78 | 79 |
| Antagonists added to pellets | | |
| None (control) | 100 | |
| *T. lignorum* | 77 | 76 |
| Glucose and *T. lignorum* | 92 | 72 |
| *Streptomyces* sp. A67 | 68 | 8 |
| *Streptomyces* sp. A67 and glucose | 96 | 4 |

[a]Percentage survival calculated in comparison with the nontreated, noninoculated controls of each series.

ably this mold stimulated the germination of the hard seeds.

*T. lignorum* was studied further as an agent for the control of damping-off of alfalfa because of the promising results reported in the literature (1, 26). Cultures of *T. lignorum* and *P. debaryanum* were grown for 7 days in tumblers ¾ filled with unground, moist, sterilized oat hulls. In one series the tumblers were inoculated with mycelial discs of *P. debaryanum*, incubated for 3 days, inoculated with the antagonist and incubated an additional 4 days. In another series *T. lignorum* was added to the tumblers first and *P. debaryanum* added subsequently. The oat hull cultures were mixed in a 1:2 ratio by volume with the ¾-in. surface layer of Plainfield sand in the pots. Alfalfa seeds were planted.

Results of this experiment appear in Table 5. Damping-off of plants grown in the presence of *P. debaryanum* only was largely pre-emergent. When *P. debaryanum* and *T. lignorum* were added to the oat hulls simultaneously, the mixture incubated 7 days and used as the inoculum, pre-emergent damping-off was slightly less than in the pots inoculated with *P. debaryanum* alone, but it was offset by greater post-emergent damping-off. Damping-off was decreased effectively only when *T. lignorum* was grown on the oat hulls for 3 days prior to the addition of *P. debaryanum*. In this instance pre-emergent damping-off was entirely absent and post-emergent damping-off was slight.

TABLE 5.—*Survival of alfalfa seedlings in soil inoculated with oat hull cultures of Trichoderma lignorum 1828 and Pythium debaryanum 285*

| Inoculum | Plants surviving 21 days |
|---|---|
| | Per cent |
| Control, oat hulls only | 100 |
| *Pythium*[a] | 50 |
| *Trichoderma*[a] | 103 |
| *Pythium* and *Trichoderma*, grown separately and mixed at time of planting[a] | 48 |
| *Pythium* and *Trichoderma*, inoculated into oat hulls simultaneously[a] | 42 |
| *Pythium* + 3 days + *Trichoderma*[b] | 48 |
| *Trichoderma* + 3 days + *Pythium*[b] | 92 |

[a]Cultures incubated 7 days.

[b]First-named culture incubated for 3 days, inoculated with the second culture and the mixture incubated an additional 4 days.

No protection against damping-off appeared when *T. lignorum*, grown on sterile oat hulls, was added to 1) Plainfield sand infested artificially with *P. debaryanum* and *P. ultimum* and to 2) naturally infested Carlisle muck. A chemical seed protectant, tetramethyl thiuram disulfide,[3] included in these experiments for comparative purposes, consistently gave complete control of damping-off in Carlisle muck. In Plainfield sand this fungicide provided good protection from *P. debaryanum* but only partial defense against *P. ultimum*.

Experiments on the protection afforded by the antagonists growing in natural Plainfield sand were extended to include 5 antagonists: *Streptomyces* spp. A66 and A67, *Bacillus* sp. B6, *P. patulum* and *T. lignorum* (Table 6). One series of pots was filled with soil containing 1 per cent alfalfa meal, another series consisted of soil to which 1 per cent ground oat straw was added, and a third series received no organic addition. *P. debaryanum* was the pathogen. Thirty ml. of an actively growing broth culture of the respective antagonist were added per 100 g. of soil used to cover the seeds. Controls consisted of pots containing soil treated with sterile broth and those inoculated with each antagonist but not containing *P. debaryanum*.

All plants grew poorly in the soil containing the alfalfa meal. A wilt condition prevailed rather than symptoms characteristic of damping-off. A majority of the plants died within a few days. During the experiment the soil reaction changed from an initial pH of 6.0 to pH 8.7-9.0. Plant mortality in this series apparently was due either to the unfavorable pH or to the accumulation of toxic levels of ammonia.

The 5 antagonists did not protect the seedlings from damping-off in this natural soil (Table 6) as consistently as they did in sterile Miami silt loam (Table 4). In the absence of added organic matter, damping-off in the nontreated pots of Plainfield sand was largely post-emergent; in the presence of oat straw, it was pre-emergent.

The temperatures at which experiments were conducted appeared to be important. In the experiment performed during July, 1949, when the temperature in the greenhouse frequently exceeded 100° F., control of damping-off by the antagonists was practically complete regardless of the presence or absence of oat straw (Fig. 7). When this experiment was repeated

[3] The product used in this investigation was Arasan, produced by E. I. Du Pont de Nemours and Co.

TABLE 6.—*Effect of 5 antagonists on the occurrence of damping-off caused by Pythium debaryanum in natural soil*[a]

| Antagonist | Seedlings surviving 14 days[b] | | | |
|---|---|---|---|---|
| | Experiment 1[c] | | Experiment 2[c] | |
| | *Pythium debaryanum* | | | |
| | Absent | Present | Absent | Present |
| | Per cent | Per cent | Per cent | Per cent |
| No organic addition: | | | | |
| None | 94 | 40 | 100 | 9 |
| *Streptomyces* sp. A66 | 100 | 99 | 95 | 24 |
| *Streptomyces* sp. A67 | 91 | 97 | 98 | 11 |
| *Bacillus* sp. B6 | 100 | 97 | 102 | 26 |
| *P. patulum* (NRRL 1952) | 95 | 96 | 94 | 23 |
| *T. lignorum* (NRRL 1828) | 98 | 100 | 100 | 15 |
| 1 per cent oat straw added: | | | | |
| None | 97 | 44 | 100 | 9 |
| *Streptomyces* sp. A66 | 99 | 100 | 90 | 12 |
| *Streptomyces* sp. A67 | 102 | 104 | 97 | 8 |
| *Bacillus* sp. B6 | 99 | 100 | 100 | 6 |
| *P. patulum* (NRRL 1952) | 99 | 91 | 83 | 5 |
| *T. lignorum* (NRRL 1828) | 104 | 101 | 99 | 7 |

[a]Plants grown in Plainfield sand. Liquid cultures of antagonists were used to moisten the soil with which the seeds were covered.
[b]Compared with the number of plants emerging in the no treated, noninoculated series of each experiment.
[c]Experiment 1 was conducted during hot weather (July, 1949); experiment 2, during cool weather (October, 1949).

during October, 1949, a similar control of damping-off occurred initially, but the protection was only temporary. Maximum greenhouse temperatures during this period seldom exceeded 80° F. Thus the end results of these experiments differed greatly (Table 6). The temporary control of damping-off produced by the antagonists in this second experiment is shown in Figure 8. The curves obtained are similar to those derived from experiments in which pots were inoculated with species of *Pythium* at various time intervals after seeding with alfalfa (Fig. 2 and 3). This relationship suggested that the antagonists delayed the infection of the seedlings with *P. debaryanum*.

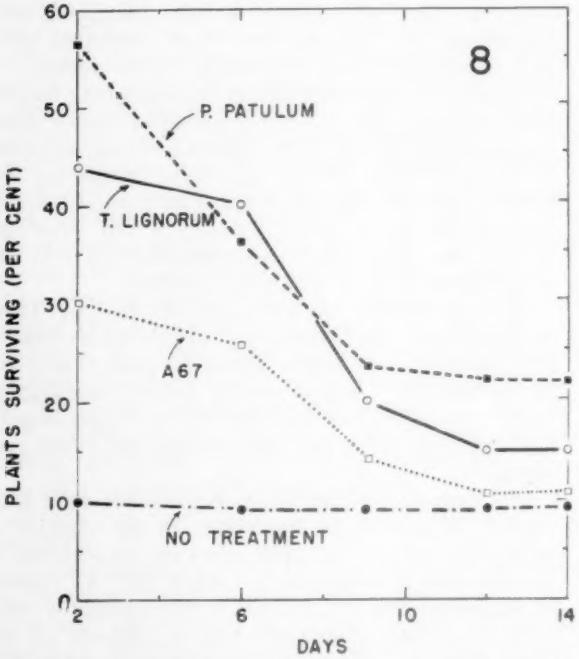



FIG. 8.—Temporary control of alfalfa damping-off by the addition of antagonistic microorganisms to natural Plainfield sand infested with *Pythium debaryanum* 285.

DISCUSSION.—Admittedly, the prerequisites for antibiotic effectiveness against damping-off, as defined in this study, were severely restrictive. Namely, a satisfactory antibiotic should 1) strongly inhibit the damping-off fungi, 2) not harm the root nodule bacteria, 3) show little or no phytotoxicity, and 4) be relatively stable in the soil. Data presented here combined with those previously reported (12) permit a comparison of the 6 antibiotics investigated with this theoretically desired antibiotic (Table 7).

Whereas each of the 6 antibiotics tested possessed some of the requirements of the theoretically desired antibiotic, none fulfilled all the criteria. Antibiotic A67, actidione, and fradicin each met to a satisfactory degree 3 of the 4 specifications. The first-named compound possessed the full range of biological specificity as shown by the inhibition of the *Pythium* species without harming the rhizobia or the alfalfa seedlings; on the other hand, its instability in soil was a major defect. Actidione and fradicin satisfied the criterion of stability in soil and also selectively inhibited the *Pythium* spp. without affecting rhizobia; but, because of their phytotoxic effects, they appeared useless for controlling damping-off of alfalfa. An antibiotic fulfilling all the above requirements remains to be found.

The possibility that adsorption of an antibiotic onto soil colloids renders it ineffective warrants consideration. Siminoff and Gottlieb (22) concluded that basic antibiotics, such as streptomycin, do not play an active biological role in the soil because they are strongly adsorbed by colloidal complexes. They found that the

TABLE 7.—*Summary of antibiotic properties involved in the control of damping-off of alfalfa*

| Culture and/or inhibitive agent | Inhibition of *Pythium* sp. | Inhibition of *Rhizobium meliloti* | Inhibition of *Medicago sativa* | Stability in soil |
|---|---|---|---|---|
| Theoretically suitable antibiotic | ++ | − | − | ++ |
| *Bacillus* sp. B6 | + | − | − | ± |
| *Streptomyces* sp. A67 | ++ | − | − | ± |
| *P. patulum* (clavacin) | ++ | ++ | ++ | ± |
| *T. lignorum* (gliotoxin) | + | + | + | − |
| Actidione | + | − | ++ | ++ |
| Fradicin | ++ | − | ++ | ++ |
| Tetramethyl thiuram disulfide (Arasan) | ++ | ++ | − | ++ |

++Inhibitive action, very strong. Stability, persists for at least 14 days.
+Inhibitive action, strong.
±Stability, persists for 1 to 4 days.
−Inhibitive action, none. Stability, persists less than 1 day.

soil exerts a considerable protective effect even upon the neutral antibiotic. Chloromycetin, which is not greatly adsorbed. Thus, 60 times as much of this antibiotic was required to inhibit *Bacillus subtilis* in soil as was required in broth (9). In the results reported here, however, soil did not noticeably protect *Pythium* species from the action of the neutral antibiotic, actidione.

The possibility of inducing continuous production of an antibiotic in soil is attractive. The temporary control of damping-off obtained in some experiments reported here may be explained in terms of the production rate of the antibiotic and the subsequent course of its decomposition (12). The added organisms undoubtedly produced enough antibiotic in the soil to inhibit the growth of *Pythium* spp. either partially or entirely for a short time. This period of semi-protection enabled some of the plants to grow through the critical period of susceptibility and thereby outgrow the pathogen. It is conjectured that microbial competition in the soil decreased the number of actively growing cells of the antagonist and hence greatly lessened its potential to produce antibiotic. Also, soil microorganisms presumably destroyed those antibiotics which were unstable in soil, whereupon the pathogen again grew freely and attacked the seedlings before they developed complete resistance. The accelerated growth rate of plants under relatively high temperatures would then advance them beyond their susceptible period to damping-off before the protective effect totally disappeared. This interpretation may explain the different results obtained between the experiments conducted during the summer as compared with those in the fall. Whereas any factor which increases the stability of an antibiotic in soil would be expected to increase its effectiveness as a control agent, any modifications of conditions should aim toward providing the best growth conditions for the plant in question. An acid soil favors the stability of gliotoxin and increases the effectiveness of *T. lignorum* (26), yet a soil sufficiently acid to assure the stability of this antibiotic is unfavorable for the growth of alfalfa.

Poor agreement was obtained between the ability of an antagonist to produce antibiotic in laboratory media and its ability to protect seedlings when added to soil. This situation was also noted in the unpublished studies of Pine (18). On the other hand, in the studies reported here the extent of disease control attributed to antagonists in the soil appeared to parallel antibiotic production *in situ*.

Since antagonistic microorganisms added to amended natural soil have been shown to produce antibiotics and to protect alfalfa from damping-off for a limited time, there remains to be found a means of prolonging this protective action. One of the most important factors in the extension of this action may be in the use of a more suitable substrate. Brian et al. (3) postulated that the production of "fungistatic organic substances" by certain *Penicillium* spp. accounted for the toxicity to mycorrhiza in Wareham heath soil. Their results suggest that prolonged production and accumulation of antibiotics in soil are possible. The results reported here indicate that both antibiotics and antagonistic microorganisms show promise in the control of damping-off of alfalfa.

## SUMMARY

Some antibiotics and microorganisms antagonistic to species of *Pythium* were employed in greenhouse experiments on the control of damping-off of alfalfa.

In these studies *Pythium ultimum* and *P. debaryanum* were the most pathogenic of 5 species of *Pythium* tested on alfalfa. The resistance of alfalfa seedlings to damping-off increased with their age. Little, if any, damping-off occurred when seedlings were inoculated with these fungi 14 to 20 days after seeding. Hence, suppression of the pathogens for 2-3 weeks after seeding was a primary objective.

Concentrations of actidione of 6.2 to 25 μg./ml. in the soil solution completely controlled damping-off of alfalfa in the greenhouse but severely stunted the seedlings. Alfalfa seeds soaked 4 hours in saturated aqueous solutions of fradicin were not harmed. Seedlings growing in the presence of 34 μg./ml. of fradicin suf-

fered severely although this concentration failed to prevent damping-off.

Inoculation of *Trichoderma lignorum* and *Streptomyces* sp. A67 into sterile soil containing 1 per cent glucose and infested with *P. debaryanum* completely prevented damping-off. *T. lignorum* added to the seed as part of a pellet protected the seedlings in sterilized soil inoculated with this pathogen. *Streptomyces* sp. A67 was ineffective when applied in this manner. Mass inoculation of *Streptomyces* spp. A66 and A67, *Bacillus* sp. B6, *Penicillium patulum*, and *T. lignorum* into infested natural soil produced variable results ranging from complete to temporary partial control. The degree of disease control obtained appeared to depend on the temperatures prevailing during the growth of the plants. High temperature favored control.

The results are discussed in terms of the criteria deemed essential for a theoretically suitable antibiotic.

DEPARTMENTS OF BACTERIOLOGY, PLANT PATHOLOGY, AND BIOCHEMISTRY
UNIVERSITY OF WISCONSIN
MADISON 6, WISCONSIN

## LITERATURE CITED

1. ALLEN, M. C., AND C. M. HAENSELER. 1935. Antagonistic action of Trichoderma on Rhizoctonia and other soil fungi. Phytopathology **25**: 244-252.
2. BEACH, W. S. 1946. Pathogenic and physiogenic damping-off. Soil Sci. **61**: 37-46.
3. BRIAN, P. W., H. G. HEMMING, AND J. C. MCGOWAN. 1945. Origin of a toxicity to mycorrhiza in Wareham heath soil. Nature **155**: 637-638.
4. BUCHHOLTZ, W. F. 1935. Relation of soil acidity to a seedling disease of alfalfa on three Iowa soils. Phytopathology **25**: 421-425.
5. BUCHHOLTZ, W. F. 1942. Influence of cultural factors on alfalfa seedling infection by Pythium debaryanum Hesse. Iowa Agric. Exp. Sta. Res. Bull. **296**: 572-592.
6. COOPER, W. E., AND S. J. P. CHILTON. 1948. Antibiosis and sugarcane root rot. (Abs.) Phytopathology **38**: 6.
7. FELBER, IRMA M., AND C. L. HAMNER. 1948. Control of mildew on bean plants by means of an antibiotic. Bot. Gaz. **110**: 324-325.
8. GOTTLIEB, D., H. H. HASSAN, AND M. B. LINN. 1950. Actidione as a plant protectant. Phytopathology **40**: 218-219.
9. GOTTLIEB, D., AND P. SIMINOFF. 1952. The production and role of antibiotics in the soil. II. Chloromycetin. Phytopathology **42**: 91-97.
10. GRANDFIELD, C. O., E. D. HANSING, AND H. L. HACKEROTT. 1948. Losses incurred in asexual propagation of alfalfa clones. Jour. Amer. Soc. Agron. **40**: 804-808.
11. GRANDFIELD, C. O., C. L. LEFEBVRE, AND W. H. METZGER. 1935. Relation between fallowing and the damping-off of alfalfa seedlings. Jour. Amer. Soc. Agron. **27**: 800-806.
12. GREGORY, K. F., O. N. ALLEN, A. J. RIKER, AND W. H. PETERSON. 1952. Antibiotics as agents for the control of certain damping-off fungi. Amer. Jour. Bot. **39**: 405-415.
13. HENRY, A. W., R. L. MILLAR, AND E. A. PETERSON. 1952. Control of covered smut of wheat by rapid seed treatment with an antibiotic. Science **115**: 90-91.
14. KERNKAMP, M. F., J. W. GIBLER, AND L. J. ELLING. 1949. Damping-off of alfalfa cuttings caused by Rhizoctonia solani. Phytopathology **39**: 928-935.
15. KREITLOW, K. W., R. J. GARBER, AND R. R. ROBINSON. 1950. Investigations on seed treatment of alfalfa, red clover, and Sudan grass for control of damping-off. Phytopathology **40**: 883-898.
16. MEREDITH, C. H., AND G. SEMENIUK. 1946. The antagonism of some species of actinomycetes in relation to soil-inhabiting plant pathogens. Report Agric. Res., Iowa Agric. Exp. Sta., Part 1: 199-202.
17. PHILLIPS, G. B., AND E. HANEL, JR. 1950. Control of mold contaminants on solid media by the use of actidione. Jour. Bact. **60**: 104-105.
18. PINE, L. 1948. Use of antibiotics and antagonistic organisms in the control of damping-off of alfalfa by Pythium debaryanum. Univ. of Wis. M.S. thesis (unpublished). Deposited in Univ. of Wis. Library.
19. ROTH, L. F., AND A. J. RIKER. 1943. Influence of temperature, moisture, and soil reaction on the damping-off of red pine seedlings by Pythium and Rhizoctonia. Jour. Agric. Res. [U. S.] **67**: 273-293.
20. RUHLOFF, MARCIA, AND J. C. BURTON. 1951. Compatibility of rhizobia with seed protectants. Soil Sci. **72**: 283-290.
21. SHARVELLE, E. G., H. C. YOUNG, JR., AND B. F. SHEMA. 1942. The value of spergon as a seed protectant for canning peas. Phytopathology **32**: 944-952.
22. SIMINOFF, P., AND D. GOTTLIEB. 1951. The production and role of antibiotics in the soil. I. The fate of streptomycin. Phytopathology **41**: 420-429.
23. SWART, E. A., A. H. ROMANO, AND S. A. WAKSMAN. 1950. Fradicin, an antifungal agent produced by Streptomyces fradiae. Proc. Soc. Exp. Biol. Med. **73**: 376-378.
24. TIMS, E. C. 1932. An actinomycete antagonistic to a Pythium root parasite of sugar cane. (Abs.) Phytopathology **22**: 27.
25. VAUGHN, J. R., J. L. LOCKWOOD, G. S. RANDWA, AND C. HAMNER. 1949. The action of actidione on plant tissue and upon certain fungi. Mich. Agric. Exp. Sta. Quart. Bull. **31**: 456-464.
26. WEINDLING, R. 1934. Studies on a lethal principle effective in the parasitic action of Trichoderma lignorum on Rhizoctonia solani and other soil fungi. Phytopathology **24**: 1153-1179.

# ROD-SHAPED VIRUS OF RADISH MOSAIC

William N. Takahashi[1]

Two morphologically different types of viruses affecting cruciferous plants are known; examples are turnip yellows virus (1) having spherical particles 22 mμ. in diameter and *Brassica nigra* Koch virus (7) having long flexuous particles 12 × 700 mμ. in length. Four serologically related strains of turnip virus 1 were found by Larson, Matthews, and Walker (5) to be associated also with long particles of undetermined lengths. In this paper a third type, relatively short rigid rod-shaped particles 25 × 105-120 mμ., is described.

The disease caused by this virus, a mosaic of cultivated radish, *Raphanus sativus* L., was described and studied by Tompkins (9) who supplied the original inoculum for the present work from several plants of mosaic White Icicle radish.

Two flats (40 seedlings per flat) of each of the hosts, White Icicle radish, *Raphanus sativus* L., Tendergreen mustard, *Brassica juncea* (L.) Coss., and Shogoin turnip, *Brassica rapa* L., were grown in the greenhouse. The seedlings of 1 flat of each set were sprinkled with carborundum powder and inoculated with a homogenate of fresh white radish leaves showing typical mosaic symptoms. Within 2 weeks plants showed symptoms of the disease, which was allowed to develop for another week; then both the mosaic and healthy plants were harvested, washed, weighed in lots of 150 gm., wrapped in aluminum foil, and frozen. A set, mosaic and healthy, of each host was treated simultaneously in the following manner.

[1]Accepted for publication August 1, 1952.

FIG. 1. Rod-shaped radish mosaic virus from Tendergreen mustard. The diameter of the polystyrene latex spheres is 260 mu.

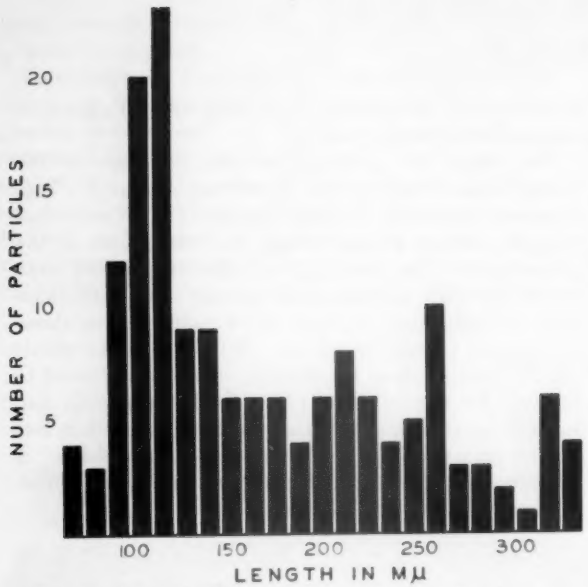



FIG. 2. Length distribution of radish mosaic virus particles.

The frozen plants were passed through a food chopper while still frozen. After the macerate had thawed, the juice was pressed from it. The press cake was extracted with sufficient volume of 0.1 M phosphate buffer of pH 7.0 to bring the combined extract to 150 ml. This was then cleared by centrifuging it for 15 min. at 10,000 × gravity. To the supernatant liquid, while stirring, 70 gm. of ammonium sulfate was added; a heavy whitish precipitate formed. After the precipitate was compacted by centrifugation for 15 minutes at 10,000 × gravity it was resuspended in 48 ml. of 0.1 M phosphate buffer of pH 7.0. The opalescent suspension was then ultracentrifuged for 1 hour at 100,000 × gravity in 4 centrifuge tubes each holding 12 ml. A definite glassy pellet was found at the bottom of each tube, including those holding the 4 healthy control samples. Another cycle of low and high speed centrifugations was run and the final 4 pellets were each resuspended in 2 ml. of 0.1 M. buffers of pH 7.0, 3 in phosphate and 1 in ammonium acetate. In this way the original virus material was concentrated from 150 gm. to 8 ml.

Forty Tendergreen mustard seedlings grown in 1 flat were inoculated with the combined phosphate suspensions. All inoculated seedlings showed mosaic symptoms within 2 weeks, indicating that active radish mosaic virus was contained in this fraction.

Electron micrograph screens were sprayed with the ammonium acetate suspensions and micrographs were taken.

All suspensions of mosaic material showed rod-shaped particles of varying lengths, together with considerable amounts of roundish particles. Similar round particles were also found in the healthy control suspensions, but no rods were present. Heating the crude extract for 5 minutes at 50° C. or pretreatment with 30 per cent alcohol did not eliminate these round particles from juice of mosaic or healthy plants.

A typical field of a preparation from mosaic mustard is shown in Fig. 1. The bar graph, Fig. 2, depicts a particle length analysis of 14 separate fields, which together contain 275 particles. The dimensions of the 2 predominant classes suggest that the typical or most frequent size of this virus is 25 × 105 to 120 mμ. The graph also shows that there is some tendency for particles of this size to aggregate end to end to form dimers and trimers.

Radish mosaic virus (2, 3, 4, 6, 8, 10) has been reported from 5 different countries and occurs on a number of local radish varieties. From symptomatology alone it is difficult to identify positively or to classify the viruses of Cruciferae. Wide host ranges and similarity of symptoms of many of the viruses of Brassica species further complicate the problem. In order to classify these viruses, it seems desirable, if possible, to determine the intrinsic characteristics of the virus, such as antigenic relations and morphology.

## SUMMARY

Rod-shaped particles were found associated with extracts of 3 different hosts infected with radish mosaic virus. These particles were 25 mμ wide and their most frequent lengths were 105-120 mμ.

No rod-shaped particles were found in extracts of healthy check plants.

Numerous spherical particles were present in extracts of both healthy and mosaic plants.

DIVISION OF PLANT PATHOLOGY
UNIVERSITY OF CALIFORNIA
BERKELEY 4, CALIFORNIA

## LITERATURE CITED

1. COSSLETT, V. E., AND R. MARKHAM. 1948. Structure of turnip yellow mosaic virus crystals in the electron microscope. Nature **161**: 250-252.
2. HAWAII AGRICULTURAL EXPERIMENT STATION. 1938. Report of the Hawaii Agricultural Experiment Station. 117 pp.
3. HINO, I. 1933. List of plants susceptible to mosaic and mosaic-like diseases. Miyazaki Col. Agric. and Forestry Bul. **5**: 111.
4. KULKARNI, G. S. 1924. Mosaic and other related diseases of crops in the Bombay Presidency. Poona Agric. Col. Mag. **16**: 6-12.
5. LARSON, R. H., R. E. F. MATTHEWS, AND J. C. WALKER. 1950. Relationships between certain viruses affecting the genus Brassica. Phytopathology **40**: 955-962.
6. OGILVIE, L. 1928. Virus diseases of plants in Bermuda. Bermuda Dept. Agric. Bul. **7**: 4-7.
7. TAKAHASHI, W. N. 1949. The morphology and host range of Brassica nigra virus. Amer. Jour. Bot. **36**: 533-535.
8. TAKIMOTO, S. 1930. On the mosaic disease of Chinese cabbage and other crucifers. Japan Hort. Mag. **42**: 5-7.
9. TOMPKINS, C. M. 1939. A mosaic disease of radish in California. Jour. Agr. Res. (U. S.) **58**: 119-130.
10. VAN DER BYL, P. A. 1931. Agriculture in the winter rainfall area. The work of an important institution. Farming in South Africa **6**: 354-358.

# EFFECT OF TEMPERATURE ON THE DEVELOPMENT OF INTERNAL CORK LESIONS IN SWEET POTATO ROOTS [1]

L. W. Nielsen

Internal cork, a virus disease of sweet potatoes, is not known to reduce the yield of affected plants. Its economic importance results from the development of necrotic lesions in diseased roots, which constitute the only reliable symptom for diagnosing the disease. Owing to the economic and diagnostic importance of the lesions, a study was made of various environmental and cultural factors that might influence their development. Data pertaining to the role of soil and storage temperature on internal cork lesion development are herein presented.

LITERATURE REVIEW.—At harvest time many sweet potato roots infected with internal cork virus have no discernible internal lesions. The proportion of freshly harvested roots that have lesions varies from year to year. On the basis of work conducted in Georgia it was postulated that environmental factors during the growing season influenced lesion development (5).

The necrotic lesions develop in diseased roots during winter storage. As the storage period progresses new lesions appear and the lesions enlarge (1, 4). A recent preliminary report showed that storage temperature influences lesion development. At a storage temperature between 78° and 80° F. new lesions formed and enlarged in diseased roots, while little change occurred in similar roots stored at 55° to 60° F. The relationship of storage temperature to lesion development was confirmed by observations of infected roots stored at 70° F. and at cooler temperatures (6).

ROOT SYMPTOMS.—External and internal root symptoms employed for studying disease response to temperature were described by Nusbaum (3). The external cavities (dimples), sometimes associated with shallow internal lesions (Fig. 1A and B), develop in growing roots. Their development appears to be due to death of tissue near the surface of the growing root. Subsequent root growth peripherally from the underlying lesion is reduced and a cavity or dimple develops.

Internal lesions were examined from thin, hand-sliced, serial cross sections of roots. Confusion sometimes arises as to the definition of a cork lesion. In numerous cases there are aggregates of small lesions separated by sound tissue. Closely aggregated lesions were arbitrarily considered to be a single lesion (Fig. 1,C).

Internal lesions vary considerably in diameter, and indirect evidence shows that they enlarge after a focus of necrosis is initiated. Necrotic tissue is readily dis-

[1] Accepted for publication July 22, 1952.
Contribution from Plant Pathology, North Carolina State College, Raleigh, N. C. Published with the approval of the Director as Paper No. 428 of the Journal series.

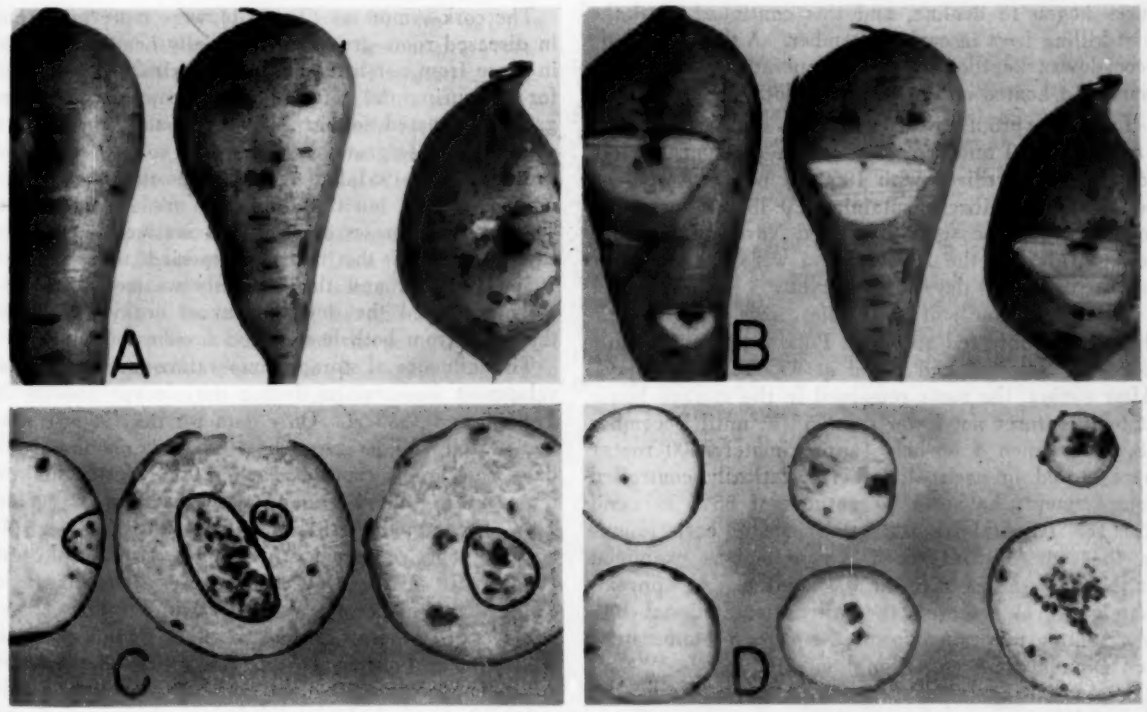



FIG. 1. Root symptoms of internal cork: A) roots having the external dimple symptom; B) same roots as A with underlying lesion exposed; C) cross-sectional slices illustrating arbitrary procedure followed in counting aggregates of small lesions, circled aggregates considered single lesions; D) slices selected to illustrate influence of temperature on lesion development after 143 days of storage at—left 55°, center 70°, right 85°.

tinguishable from sound tissue; affected tissue is irregular in shape. The lesions usually have their greater dimension paralleling the axis of the root. The cross sectional diameter of the largest lesion in individual roots can be used for measuring the progress of disease development.

The number of lesions per diseased root varies. At harvest, many roots have no lesions; others have several. After prolonged storage diseased roots may have 20 or more lesions, and it is difficult to distinguish single lesions. In roots having numerous lesions, small lesions in close proximity probably coalesce as they enlarge. Therefore, the number of discrete lesions in a root is only relative and under certain conditions changes with time. However, the number of lesions in individual roots is another basis for measuring the development of internal cork.

MATERIALS AND METHODS.—The influence of soil temperature on lesion development in growing roots was tested by artificially heating field plots with electric cables during the growing seasons of 1950 and 1951. Heating cables 60 ft. in length were placed in furrows opened for receiving fertilizer. Each plot was approximately 30 ft. long and the cable was doubled back giving 2 parallel areas of heated soil extending the length of the plots. The cables were about 5 to 6 in. apart in the bottom of the fertilizer furrow and 5 to 6 in. below the ridge of the row. The temperature controlling thermostat was set at 80° F. in 1950, and 85° F. in 1951. Artificial heat was supplied each year about mid-September after the high summer temperatures began to decline, and was continued until the first killing frost in early November. A thermographic record was kept of the soil temperature in the root zone of a heated and non-heated plot.

Infected sprouts were used for setting the plots June 28, 1950 and July 6, 1951 in plots of Norfolk sandy loam fertilized with 1000 lb. per acre of 3-9-9 commercial fertilizer containing 20 lb. of borax per ton. The potatoes were harvested November 7 and 8 respectively, for the 2 years, and were examined for lesions within 30 days after harvest.

Roots used for storage studies were likewise produced from infected sprouts. Potatoes of marketable size were selected and cured at 85° F. for 10 days. After curing, the roots remained in the storage house at temperatures not lower that 55° F. until December 18, 1950, when 4 bushels (approximately 400 roots) were stored in each of 3 thermostatically controlled compartments having temperatures of 55°, 70° and 85° F. To establish the prevalence of cork lesions in the experimental roots, a sample was examined at the time the experiment was started. At approximately 6-week intervals thereafter an additional 100-root sample was taken from each storage temperature and examined for cork symptoms.

The following criteria were used for recording the data: dimples per root (soil temperature studies), per cent roots with lesions, lesions per root and the diameter of the largest lesion in each root (in inches or fractions thereof). Lesion diameter data were used for calculating the cork index. The method of classifying the measurements and calculating the index was that used by Nusbaum (4) except that the number of classes and the range of lesion sizes in each class differed slightly. The following classes and lesion diameters were used: 0) free of lesions; 1) lesion up to ⅛ in.; 2) ⅛ to ¼ in.; 3) ¼ to ½ in.; 4) ½ to ¾ in.; and 5) ¾ to 1 in. or larger. The cork index was computed as follows: $\text{Cork index} = \frac{S(1n)+(2n)-(5n)}{N}$, where n equals the number of roots in each class; and N, the total number of roots in a given sample.

TABLE 1.—*The prevalence of internal cork symptoms in marketable sweet potato roots grown in heated and nonheated soil. Experiment performed in 1950 and 1951*

| Treatment | Dimples per root | Lesions per root | Cork index | Per cent roots with lesions |
|---|---|---|---|---|
| | | *1950* | | |
| Heat | .36 | .72 | .81 | 31.8 |
| No heat | .07 | .16 | .27 | 10.4 |
| | | *1951* | | |
| Heat | .18 | 2.31 | 1.34 | 62.82 |
| No heat | .08 | 1.51 | .97 | 50.27 |

EXPERIMENTAL RESULTS.—The soil temperature experiment was performed at the McCullers Experiment Station. Artificially heated plots were approximately 10 to 15 degrees warmer (Fig. 2) than the non-heated plots during the later part of the growing season.

The cork symptoms (Table 1) were more numerous in diseased roots grown in artificially heated soil than in those from non-heated soil. For all categories used for measuring the internal cork symptom, the roots grown in heated soil in 1950 had 3 to 4 times more cork than those grown in non-heated soil. Roots from heated soil in 1951 had more lesions than roots from non-heated soil, but the differences are not significant. The growing season of 1951 was warmer during the early weeks of the treatment period than that of 1950 (Fig. 2), and the naturally warmer soils possibly increased the development of corky lesions in the roots from both heated and non-heated plots.

The influence of storage temperature upon lesion development was studied during the storage seasons of 1949-50 and 1950-51. Only data for the 1950-51 storage season will be given here as the previous year's data were briefly reported earlier (2). At the highest temperature (85° F.) many of the small roots (about 1 in. in diameter) shriveled and were useless. At 55° the roots remained firm and the last sample was cut July 18, 1951. Data from the experiment are in Table 2.

Storage temperature had a marked influence on lesion development. At the 2 warmer temperatures additional roots developed lesions, and the lesions enlarged (cork index) and became more numerous than in similar roots stored at 55° F. At 85° F., significant increases in lesion development were

TABLE 2.—*Influence of storage temperature on the development of internal cork lesions in Porto Rico sweet potato roots. All roots produced from infected sprouts*

| Sampling date | Storage Temp.-°F. | Lesions per root | Per cent roots with lesions | Cork index |
|---|---|---|---|---|
| 12-18-50[a] | ---- | 1.1 | 43.9 | 0.56 |
| 1-23-51 | 55 | 1.2 | 47.0 | 0.74 |
| | 70 | 1.9 | 55.0 | 1.04 |
| | 85 | 2.0 | 61.6 | 1.14 |
| 3-20-51 | 55 | 1.0 | 44.0 | 0.88 |
| | 70 | 4.7 | 90.0 | 2.10 |
| | 85 | 6.4 | 90.0 | 2.59 |
| 5-10-51 | 55 | 1.0 | 43.0 | 0.90 |
| | 70 | 4.9 | 93.8 | 2.61 |
| | 85 | 9.4 | 100.0 | 3.60 |
| 7-18-51 | 55 | 1.3 | 45.5 | 0.61 |
| L. S. D. 1% | | 1.9 | 12.7 | 0.57 |

[a]Date on which roots were placed at various storage temperatures.

measurable after 5 weeks of storage. These differences became more pronounced as the storage season progressed (Fig. 1D). The data for roots held at 55° F. are of particular interest. In these potatoes the number of roots having lesions and the lesions per root did not change over the 7-month storage period. There was evidence, however, that the lesions present in the roots at the time the experiment started slowly increased in size (cork index) during this storage period.

DISCUSSION.—Soil temperature is an important factor in the development of lesions in the growing root. This factor may partially account for the variation observed in the prevalence of cork lesions from year to year in freshly harvested roots. There were more roots with lesions in 1951 than in 1950, and this is correlated with naturally warmer soils in 1951 (Fig. 2). Other factors also influence the development of lesions in the growing root. Some of these will be discussed in a later paper.

Storage temperature is a major factor in the development of lesions in infected roots. Data from roots held at 55° F. suggest a procedure for minimizing economic losses from this disease. When harvested, many infected roots have no lesions, and the lesions present are generally small. Storing such roots at 55° F. after curing practically stops further lesion development.

During the progress of the experiments on root storage, 1 bushel of diseased roots was held at approximately 94° F. Some of these roots were held at this temperature for 108 days. After this storage period all the roots (23) had lesions, but the number of lesions and their size were less than those in potatoes stored at 85° for the same interval of time. Owing to the small sample, this response to the higher temperature cannot be critically compared with that of roots stored at 85° F.

Since finding that warm storage temperatures accelerate the development of lesions in diseased roots, all experimental and commercial samples of roots are stored at 80° to 85° F. for 3 to 4 months so that a better appraisal of diseased roots can be obtained. Shrinkage from sprouting and water loss also increases at the warmer storage temperatures. Small potatoes approximately an inch or less in diameter frequently shrivel and have to be discarded. The smallest loss from shrinkage was experienced when larger roots were used.

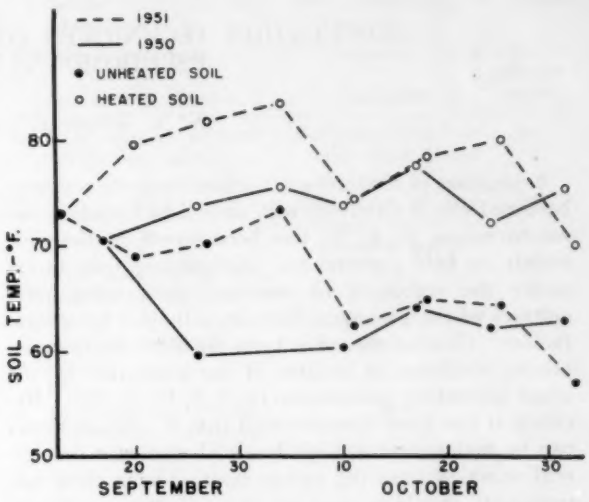



FIG. 2. Relative soil temperature of heated and unheated plots for 1950 and 1951. For both years the heat was turned on about mid-September. Temperature plotted as weekly average.

SUMMARY

Sweet potato roots infected with the internal cork virus were grown in soils artificially heated from mid-September until early November, and more necrotic lesions developed in them than in similar roots grown in non-heated soils.

Storage temperature markedly influences lesion development in diseased roots. Additional infected roots develop lesions and the lesions become larger and more numerous at storage temperatures of 70°, 85° and 94° F. In contrast, no additional lesions developed in roots stored at 55° F.; however, there was evidence that the lesions present enlarged slowly over a 7-month period.

NORTH CAROLINA STATE COLLEGE
RALEIGH, NORTH CAROLINA

LITERATURE CITED

1. MARTIN, W. J. 1949. Internal cork of sweet potatoes. Annual Report Louisiana Agr. Expt. Sta. 1947-48: 98.
2. NIELSEN, L. W. 1951. Influence of temperature on the development of internal-cork symptoms in sweet potato roots. Phytopathology **41**: 28.
3. NUSBAUM, C. J. 1946. Internal cork, a new disease of sweet potato of unidentified cause. Phytopathology **36**: 18-23.
4. NUSBAUM, C. J. 1950. Internal cork of sweet potatoes. South Carolina Agr. Expt. Sta. Bul. 381.
5. RANKIN, H. W. 1950. Studies of internal cork of sweet potatoes. Phytopathology **40**: 790-791.
6. WILCOX, MARGUERITE S. AND B. D. EZELL. 1951. Storage temperature and the development of internal cork in sweet potatoes. Phytopathology **41**: 477-479.

# INOCULATION TECHNIQUES FOR EVALUATING RESISTANCE TO PSEUDOMONAS SOLANACEARUM [1]

N. N. Winstead [2] and Arthur Kelman [3]

Evaluation of resistance in various crops to southern bacterial wilt or Granville wilt, caused by *Pseudomonas solanacearum* E. F. S., has been based in the past mainly on field experiments. Greenhouse tests to expedite the screening of resistant lines, using pure cultures of the pathogen, have been limited by several factors. Chief of these has been the difficulty in maintaining virulence in isolates of the bacterium by the usual laboratory procedures (3, 7, 8, 9, 11, 13). Recently it has been demonstrated that *P. solanacearum* can be maintained at high levels of virulence for several years by covering potato-dextrose-agar slant cultures with sterile mineral oil (5). Critical studies of the influence of environmental factors on disease development (1, 2, 14) have also indicated that optimum conditions for pathogenesis could be provided in the greenhouse by maintaining relatively high soil moisture and temperature levels. Although a number of different techniques have been used for inoculations with pure cultures of *P. solanacearum* (11), neither standardization nor evaluation of these techniques for use in large scale greenhouse inoculations have been attempted. In connection with the expansion of a program for development of tomato (*Lycopersicon esculentum* Mill.) lines with resistance to southern bacterial wilt, such a standardization of greenhouse procedures was necessary. Information on the relative levels of resistance in certain tobacco (*Nicotiana tabacum* L.) lines was also needed to supplement field data.

The investigations reported in this paper, therefore, had the following objectives: 1) to evaluate inoculation techniques for the most effective testing of large numbers of plants under greenhouse conditions, 2) to determine the procedure which would insure uniform and rapid development of bacterial wilt without overcoming the inherent resistance of lines being tested, and 3) to evaluate certain factors contributing to variation in results. Preliminary reports on certain phases of these studies have been made (4, 15).

MATERIALS AND METHODS.—In the majority of tests tomato plants were used: the susceptible tomato varieties included Marglobe and Louisiana Pink; the resistant types included three $F_5$ and two $F_6$ lines.[4] The $F_5$ lines (R2, R4, and R5) and one $F_6$ line (L1) were selections from the cross Louisiana Pink × [(Louisiana Pink × T-414)—2]. The second $F_6$ line (L2) was a selection from the cross Rutgers × Mulua. Mulua and T-414 are types of *L. esculentum* from Guatemala and Puerto Rico respectively. The following susceptible varieties of other host plants were also tested: Irish Cobbler potato (*Solanum tuberosum* L.), North Carolina 4 peanut (*Arachis hypogaea* L.), and Variety 402 tobacco. One resistant peanut variety, Schwarz 21,[5] was used. This variety was originally developed in Java (10) for resistance to bacterial wilt. The wilt resistant tobacco varieties used included Oxford 26 and the Dixie Bright varieties 27, 101 and 102 (6). The last 2 varieties also have resistance to the black shank disease caused by *Phytophthora parasitica* (Dast.) var. *nicotianae* (Breda de Haan) Tucker.

In most experiments individual plants were grown in soil in clay pots. In certain tests with tomato the plants were grown in soil in wooden flats (14 in. × 21 in. × 4 in.). The steamed soil mixture used consisted of 8 parts sandy-loam soil, 2 parts sand and 1 part peat moss.

Stock cultures of pathogenic isolates of *P. solanacearum* were maintained in test tubes on potato-dextrose agar slants covered with sterile mineral oil (5). Inoculum was usually obtained from 3- to 5-day-old cultures grown on potato-dextrose agar in 400 ml. flat-sided pharmaceutical bottles. Bacterial suspensions were made by adding 100 ml. of sterile tap water to each bottle culture and gently agitating until the bacterial growth was suspended in the water. The suspension from each bottle culture was then diluted by the addition of 300 ml. of water. Infested soil from field plots at Oxford, N. C., and diseased plant debris were also used as sources of inoculum in certain preliminary experiments.

Inoculations with pure cultures were usually made by 1) forcing a sharp needle into the stem through a drop of bacterial suspension placed in the axil of the second or third expanded leaf below the stem apex, 2) cutting the lateral roots with a scalpel along one side of the plant to a depth of approximately 4 cm. and pouring 10 ml. of a bacterial suspension over the severed roots, or 3) dipping the roots in a bacterial suspension. The first 2 procedures will be referred to in the text as the stem-puncture or stem-inoculation technique and the root-injury or root-inoculation technique respectively.

[1]Accepted for publication July 22, 1952.

Published with approval of the Director as Paper No. 431 of the Journal Series of the North Carolina Agricultural Experiment Station.

[2]Formerly graduate research assistant in Plant Pathology, North Carolina State College, Raleigh, North Carolina.

[3]Assistant Professor of Plant Pathology, North Carolina State College, Raleigh, North Carolina.

The authors gratefully acknowledge the advice and assistance of Drs. D. E. Ellis, L. W. Nielsen and J. H. Jensen of the North Carolina Agricultural Experiment Station during the course of the investigation and in the preparation of the manuscript.

[4]Tomato lines developed at N. C. State College in a cooperative breeding project conducted by the Departments of Horticulture and Plant Pathology.

[5]Appreciation is expressed to Dr. W. C. Gregory, Agronomy Department, N. C. State College for providing the Schwarz 21 seed.

Disease readings were usually made at weekly intervals by classifying each plant according to one of the following numerical grades: no symptoms, O; 1 leaf partially wilted, 1; 2 or 3 leaves wilted, 2; all except the top 2 or 3 leaves wilted, 3; all leaves wilted, 4; and dead, 5. Readings were converted to disease indices by the following procedure: the number of plants in each symptom category was multiplied by the corresponding numerical grade and the products added. The summation was converted to a disease index value by dividing by the maximum numerical grade for the given number of plants and multiplying by 100. Inoculated plants were sectioned at the end of each test and examined for vascular discoloration characteristic of invasion by *P. solanacearum*. Reisolations from inoculated plants were also frequently made.

Following inoculations, soil moisture was maintained at high levels and temperatures were held at or above 30° C. during the day and 20° C. during the night. During the winter months artificial light was used to increase day length to 15 hours.

The treatments were arranged in either randomized-block or split-plot designs. The data obtained were analyzed statistically.

RESULTS.—*Relative effectiveness of different inoculation techniques on susceptible host plants.*—In preliminary experiments naturally infested field soil and soil containing plant debris were compared with pure cultures of the pathogen as sources of inoculum. Development of wilt symptoms was frequently erratic in Marglobe tomato, Variety 402 tobacco, and Irish Cobbler potato plants when grown in pots containing naturally infested soil or soil with diseased plant material. In several instances test plants showed no symptoms after a growth period in infested soil of 4 to 5 weeks. In contrast these susceptible host plants were usually all killed within 3 to 5 weeks if pure cultures and the root-injury technique were used. In addition to the difficulty in obtaining uniform disease development, it was impossible to standardize inoculum dosage when diseased plant debris or infested field soil were used. For these reasons valid comparisons between successive experiments could not be made, and tests using these sources of inoculum were discontinued.

In the initial experiments with pure cultures, young succulent Marglobe tomato, Variety 402 tobacco, and Irish Cobbler potato plants gave uniformly high disease readings with the stem-puncture or root-injury techniques. Table 1 presents the results of a typical experiment in which 4-week-old Irish Cobbler potato plants with 8 to 10 expanded leaves were inoculated by various techniques.

Symptoms of wilt were first evident in those plants inoculated by puncturing the stem through a drop of bacterial suspension placed in the axil of the third leaf below the stem tip. At the end of 15 days all plants that were inoculated by the stem-puncture procedure noted above, or by the root-injury or root-dipping techniques, were either dead or dying. Disease development was less rapid in plants inoculated near the stem tip with bacterial slime scraped from surface of cultures growing on potato-dextrose agar plates. Inoculation by pouring the bacterial suspension over the soil without injuring the roots was not an effective procedure.

TABLE 1. *Relative severity of bacterial wilt in Irish Cobbler potato plants 11 days after inoculation by various procedures*

| | Inoculation technique | Inoculum | Disease index[a] |
|---|---|---|---|
| 1. | Stem puncture at third node below apex | Bacterial slime from agar slant placed at node | 66 |
| 2. | Do | One drop of bacterial suspension placed in leaf axil | 94 |
| 3. | Stem puncture at first node above soil line | Do | 76 |
| 4. | Roots cut along one side and bacterial suspension poured over soil | 10 ml. of bacterial suspension | 82 |
| 5. | Roots not injured; bacterial suspension poured over soil | Do | 1.2 |
| 6. | Roots dipped | Bacterial suspension | 82 |

[a]Least significant difference at 0.05 level = 15.6; at 0.01 level = 20.6.

Similar results were also obtained in tests with Marglobe tomato seedlings when succulent 4- or 5-week-old plants were used. If 7- or 8-week-old plants were used, inoculations by stem-puncture at the first node above the ground were not as effective as those at the second or third node below the stem tip. Also more uniform results in stem-inoculations were obtained by use of a drop of suspension rather than a small amount of bacterial slime. If care was exercised in making the puncture into the stem through a drop of suspension, the inoculum was usually drawn rapidly into the vascular tissue; presumably more rapid distribution of the pathogen resulted.

Although inoculation by dipping roots in a bacterial suspension was usually very effective, the severe injury to the root system associated with this technique frequently caused a temporary stunting of check plants, with the stunting more pronounced on larger plants. This cessation of growth was not evident in check plants wounded by puncturing the stem or cutting the secondary roots along one side of the tap root. Because of the stunting further tests using the root-dipping technique were discontinued.

*Relation of inoculation court to disease severity in resistant and susceptible plants.*—In inoculation experiments with susceptible tomato, tobacco, and potato seedlings there was no evidence of a difference between root and stem tissues with respect to resistance to invasion by *P. solanacearum*. However, in connection with host range studies, T. E. Smith (12) observed that certain legumes were resistant to bacterial

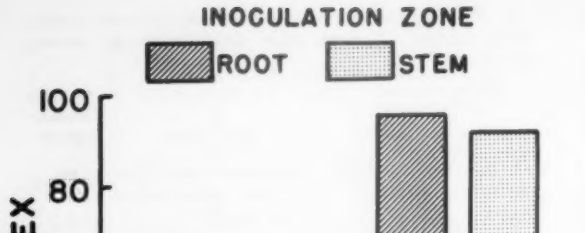



FIG. 1. Relative disease severity in resistant Oxford 26 and susceptible Variety 402 tobacco plants 28 days after inoculation by the root-injury or stem-puncture techniques.

wilt under field conditions where infection occurs usually through roots. The same legumes were susceptible when stem-inoculation tests were made in the greenhouse. These findings suggested that there might also be a difference in susceptibility between root and stem tissues of resistant varieties in other crops. The existence of a difference of this type would naturally influence the selection of a valid inoculation technique. To investigate this possibility, root and stem-inoculations were made initially on tobacco plants of the resistant Oxford 26 variety and the susceptible Variety 402. At the time of inoculation the 8-week-old plants were about 32 cm. in height and had 7 to 8 expanded leaves. Results of this experiment based on readings taken 28 days after inoculation are presented in Figure 1.

With the resistant variety, stem-inoculation resulted in a significantly higher disease reading than root-inoculation, whereas with the susceptible Variety 402 there was no real difference in effectiveness between the 2 procedures. Disease severity was significantly lower in Oxford 26 than in Variety 402 even following stem-inoculation.

Similar results were also obtained when stem- and root-inoculations were made on the wilt resistant Dixie Bright varieties 27, 101 and 102.[6] At the time of inoculation in this test the 8-week-old plants were about 28 to 36 cm. in height with 6 to 8 expanded leaves. Higher disease readings were obtained by stem-inoculation in the Dixie Bright varieties than by root-inoculation. In Variety 402 wilt symptoms first appeared in stem-inoculated plants, but when the final reading was taken at the end of 6-weeks real differences were not evident between the plants inoculated by either method. There were significant differences in relative resistance between the Dixie Bright varieties, Dixie Bright 27 being the least resistant and Dixie Bright 102 most resistant. Under field conditions it had not been possible to demonstrate that differences between the varieties were significant. In many of the Dixie Bright 102 plants which showed no leaf symptoms there was some slight vascular discoloration near the inoculation courts, indicating restricted development of the pathogen following initial invasion of the suscept tissue.

The root-injury technique was also compared with the stem-puncture technique using 2 peanut varieties, susceptible North Carolina 4 and resistant Schwarz 21. At the time of inoculation the 2-week-old peanut plants were about 14 cm. in height with 5 to 6 expanded leaves. Final readings were taken 21 days after inoculation when most of the N. C. 4 plants inoculated by the stem-puncture technique were dead or dying. Disease readings in N. C. 4 plants inoculated by the root-injury technique, however, were lower than readings from stem-inoculated plants. Although this relationship was also true for the resistant Schwarz 21 plants, the levels of infection in this variety were extremely low. None of the Schwarz 21 plants inoculated by root-injury technique had developed advanced wilt symptoms at the time of the final reading. When the stems and tap roots of inoculated Schwarz 21 plants showing no foliage symptoms were sectioned, vascular discoloration was usually restricted to inoculation points. Similar results were obtained when this experiment was repeated.

To determine the reaction of susceptible and resistant tomato plants to stem and root inoculations, Marglobe and 2 resistant $F_6$ tomato lines (L1 and L2) were compared. At the time of inoculation the 6-week-old plants were about 28 to 32 cm. high with 8 to 10 expanded leaves. Results obtained 21 days after inoculation are summarized in Figure 2.

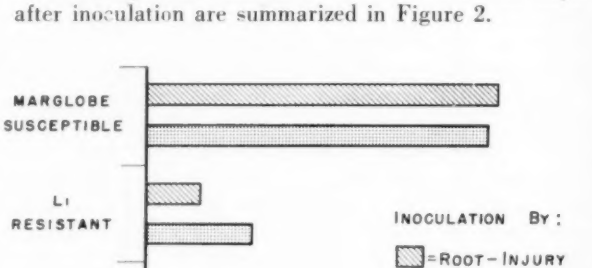



FIG. 2. Severity of bacterial wilt in susceptible and resistant tomato lines 21 days after root- or stem-inoculations.

[6]The authors are indebted to Dr. G. B. Lucas for his assistance in the completion of this experiment.

The results were in essential agreement with those obtained with resistant and susceptible tobacco varieties. There was an interaction between points of inoculation and lines due to the high susceptibility of Marglobe plants regardless of inoculation point. In contrast, each of the $F_6$ lines was more susceptible to stem-inoculations than to root-inoculations. Furthermore, the $F_6$ plants were more resistant than the Marglobe regardless of inoculation method. In 2 additional experiments in which Marglobe was compared with 8 resistant $F_5$ tomato lines, results similar to those presented in Figure 2 were obtained.

*Relationship of age of resistant and susceptible tomato plants to severity of bacterial wilt.*—In the tests comparing relative effectiveness of stem-puncture and root-injury techniques on resistant and susceptible tomato lines, plants were usually inoculated when they were about 6 weeks old, 28 to 32 cm. in height and with 8 to 10 expanded leaves. In order to determine whether the results obtained in the preceding experiments could be duplicated with younger and smaller plants, the following test was completed using susceptible Marglobe and resistant $F_6$ (lines L1 and L2) plants. The 4-week-old seedlings were 8 to 10 cm. tall with 3 to 4 expanded leaves when inoculated by either the stem-puncture or root-injury techniques. Disease development was extremely rapid in the Marglobe seedlings and only slightly less rapid in the resistant plants. Final disease readings taken 21 days after inoculation revealed no real difference between the 2 inoculation procedures in either resistant or susceptible plants. The resistant plants inoculated at this early stage of development succumbed to infection almost as rapidly as the susceptible plants.

To investigate further the relationship of age to disease development, an experiment was designed to appraise the reaction to root and stem inoculations of susceptible and resistant tomato plants in 3 age groups. The Marglobe and resistant $F_6$ seedlings (lines L1 and L2) used for this test were obtained from plantings which were made in flats on 3 successive dates. Ten days after emergence the seedlings were transplanted to 4-inch clay pots. At the time of inoculation the mean heights of plants 4, 6 and 7 weeks old were 8, 24 and 38 cm. respectively. The results, based on readings taken 21 days after inoculation, are presented in Figure 3.

In the analysis of the results significant interactions were obtained between disease indices of 1) different tomato lines and the point of inoculation, 2) different lines and ages of plants and 3) different ages and the point of inoculation. In 4-week-old Marglobe and L1 and L2 plants, relatively high disease readings resulted from either stem-or-root inoculations. The data confirmed the results of the preceding test indicating that small young plants of resistant lines were highly susceptible when inoculated by either the stem-puncture or root-injury techniques. However, there was a marked difference in relative resistance between 4-week-old and 6-week-old plants of the resistant lines.

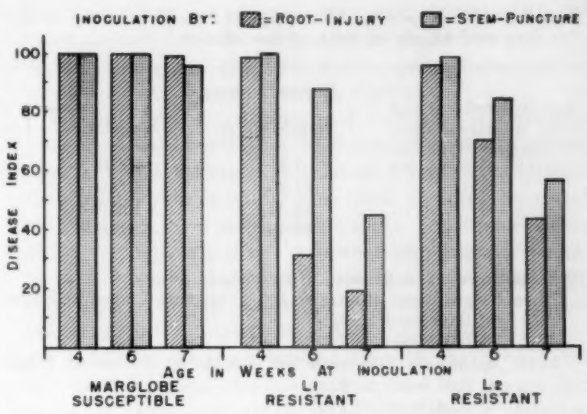



FIG. 3. Severity of bacterial wilt in susceptible Marglobe and resistant $F_6$ (L1 and L2) plants as influenced by age of plants and point of inoculation.

In addition, 6-week-old and 7-week-old plants of the resistant lines inoculated by the root-injury technique showed greater resistance than those inoculated by the stem-puncture technique. There were no significant differences in the disease indices for Marglobe plants irrespective of the age or the point of inoculation.

An additional experiment was completed to determine the relationship of age and height to disease development in susceptible Marglobe, Louisiana Pink and resistant $F_5$ plants. In this test all plants were transplanted to pots on successive dates and inoculated only by the root-injury technique. Plants within each age group were divided into 3 sizes. At the time of inoculation plants were 4, 5, 6, and 8 weeks old. The 8-week-old plants were somewhat pot-bound and starting to bloom; plants of other ages were still succulent at the time of inoculation. The results based on readings taken 21 days after inoculation are in Table 2.

There were no significant differences in mean disease indices between sizes within 1 age group for any 1 line. There was an interaction between ages and varieties. There were no significant mean differences in disease indices between Marglobe, Louisiana Pink, R2 or R4 plants inoculated when 4 weeks old. At this age only the R5 plants showed evidence of resistance. Wilt severity in resistant lines decreased as age at the time of inoculation increased. Marglobe and Louisiana Pink plants 8 weeks old at the time of inoculation had lower disease readings than younger plants. However, the plants at this age were somewhat pot-bound and the less favorable growing conditions may have contributed to an apparent increase in resistance.

Increased resistance in the resistant lines was apparently associated with age rather than with size of plants. At the time of inoculation in this experiment it was noted that some of the 5-week-old plants were taller than some of the 6-week-old plants. A study was made of possible relationship between height and disease severity in these plants with the following results. Six-week-old R4 plants with a mean height of 24 cm.

TABLE 2. *Disease severity*[a] *in resistant $F_5$ lines and susceptible Marglobe and Louisiana Pink varieties as influenced by plant age and height at time of inoculation.*

| Age in weeks at the time of inoculation | Range in mean height in cm. | Disease index[b] | | | | |
|---|---|---|---|---|---|---|
| | | Marglobe | Louisiana Pink | Line R2 | Line R4 | Line R5 |
| 4 | 9-11 | 100.0 | 100.0 | 93.0 | 92.0 | 78.0 |
| 5 | 21-25 | 100.0 | 100.0 | 62.0 | 60.4 | 66.4 |
| 6 | 28-31 | 100.0 | 100.0 | 58.0 | 47.4 | 38.4 |
| 8 | 49-59 | 85.0 | 58.0 | 5.0 | 4.0 | 9.4 |

[a] Readings taken 21 days after inoculation.
[b] Least significant difference for comparing 2 ages in 1 line.
at 0.05 level = 11.0
at 0.01 level = 14.8
Least significant difference for comparing 2 lines at 1 age.
at 0.05 level = 10.4
at 0.01 level = 14.0

at the time of inoculation had a mean disease index of 39.0, whereas 5-week-old plants 26 cm. in height had a mean disease index of 54.0. Similarly in line R5, 6-week-old plants with an initial mean height of 23 cm. had a mean disease index of 37.0, whereas 5-week-old plants with a mean height of 26 cm. had a mean disease index of 61.0. These differences in disease indices were significant at the 1 per cent level.

*Effect of stand density on disease development.*—In the greenhouse evaluation of tomato breeding lines for resistance to bacterial wilt, it was desirable to grow and inoculate the maximum number of plants in each line. To determine whether it would be possible to grow and inoculate relatively large numbers of seedlings per flat with dependable and comparable results, a randomized block experiment with 3 replications was designed. Each flat was marked off into 9 rows. Plant density was varied by planting from 10 to 75 seeds of the Marglobe variety in each row. Seeds were planted so as to insure stands of evenly spaced plants. After emergence, seedlings were thinned to 50, 40, 30, 20, 10 and 5 plants per row, the stand density thus ranging from 450 to 45 plants per flat. Root-inoculations were made when the plants were in the 4- to 6-leaf stage of growth and about 10 to 12 cm. tall. No real differences in disease readings were obtained between any of the plant population groups ranging from 45 to 450 plants per flat. The disease indices after 21 days ranged from 99.5 where there were 450 plants per flat, to 100 for 180, 90 and 45 plants per flat. This experiment was repeated with comparable results.

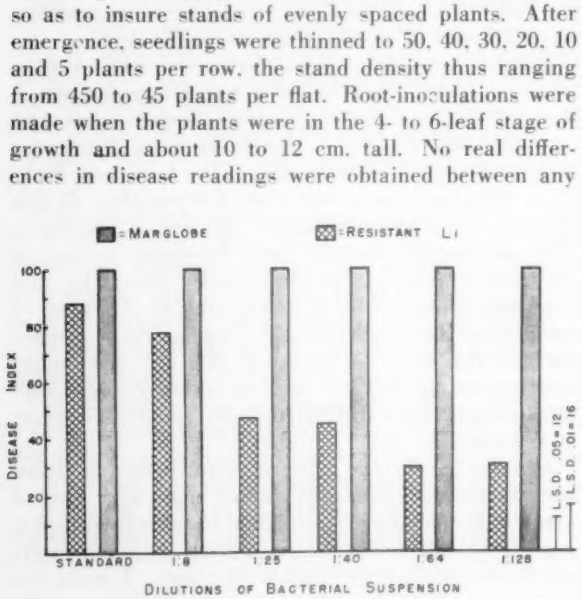



FIG. 4. Relationship between concentration of bacterial suspensions and disease severity in resistant L1 and susceptible Marglobe tomato plants.

*Influence of inoculum concentration on the severity of bacterial wilt in resistant and susceptible tomato lines.*—In the preceding experiments the bacterial inoculum was standardized by suspending the bacteria from 1 bottle culture in 400 ml. of water. The possibility existed that variations in inoculum concentration would contribute to differences in disease development in different inoculation experiments. In a preliminary trial there were no evident differences in the incidence of wilt in Marglobe tomato and Irish Cobbler potato plants when inoculated with either the standard bacterial suspension or a 1:25 dilution of the standard.

To investigate the effect of inoculum concentration on disease development in young susceptible and resistant tomato plants, an experiment was completed using Marglobe and L1 tomato plants. A series of dilutions of the standard bacterial suspension was made. The following turbidity readings for these dilutions were obtained with the Fisher electrophotometer (Filter #425) 1:8 dilution, 15.0; 1:25, 5.0; 1:40, 3.0; 1:64, 1.7 and 1:128, 0.6. At the time of inoculation by the root-injury technique, the 4-week-old plants were about 8 to 10 cm. tall with 4 to 5 expanded leaves. The data recorded 21 days after inoculation are summarized in Figure 4. There were no differences in disease indices of Marglobe plants due to inoculum concentration. However, there was a marked decrease in disease incidence in the L1 plants as the inoculum concentration decreased. The results of this experiment indicated the possibility of differentiating between resistant and susceptible lines by inoculating relatively small young plants with dilute bacterial suspensions.

In 2 additional tests the turbidity of the bacterial suspensions was adjusted by use of the Fisher electrophotometer to levels equal to those noted in the experiment above. The results obtained in these tests were essentially similar to those obtained in the preceding experiment.

DISCUSSION.—On the basis of these studies it was concluded that the root-injury technique would probably be the best procedure for evaluating resistance to *P. solanacearum* in tomato and tobacco under greenhouse conditions. Although stem-inoculations consistently produced higher wilt readings in resistant plants, greatest differentiation between resistant and susceptible plants was obtained by root-inoculations. For differentiation of lines with very high levels of resistance the stem-inoculation procedure may have certain advantages. Introduction of the inoculum directly into the stem did not completely overcome the inherent resistance of the more resistant tomato, tobacco and peanut plants. However, in extensive greenhouse tests, exclusive use of the stem-puncture technique might result in elimination of certain lines which have field resistance. This viewpoint is supported to some extent by the work of T. E. Smith (11). In studies on the host range of *P. solanacearum* Smith found that soybean, velvet bean, and cowpea seedlings were susceptible when stem-inoculations were used. The same hosts never contracted the disease when planted in the field in heavily infested soil.

Although no specific studies were directed towards a determination of the nature of resistance, certain observations suggest that establishment of the pathogen is determined largely by those characteristics of the suscept which inhibit rapid multiplication of the organism and prevent circumferential rather than longitudinal spread. Necrosis of vascular tissues extending around the stele from the point of inoculation was usually limited in the more resistant hosts. However, in many of these plants isolated discolored vascular strands were observed which extended some distance up the stem from the points where initial invasion had occurred in the roots. Following root-inoculations the bacterium could be isolated from the vascular tissue of stems of resistant tomato and tobacco plants which showed no external wilting symptoms and relatively little vascular discoloration. Apparently there is no mechanism for prevention of longitudinal spread in the resistant types. In susceptible host plants all vascular tissues as well as the pith in the region of inoculation were usually dark brown to black and showed marked necrosis within 10 days to 2 weeks after inoculation.

If it is assumed that root- or stem-inoculations provide equal opportunity for ingress of the pathogen, resistance to invasion by the bacterium is apparently greater in the root tissues than in the stem tissues. Although the physiological characteristics of resistant plants may be the primary factors involved, it is recognized that there are inherent differences in the 2 inoculation techniques which may also contribute to this difference. In the stem-puncture procedure, although there is only 1 point of entry, the drop of bacterial suspension is probably drawn rapidly into the xylem. Presumably the bacteria have unrestricted opportunity for movement and multiplication in the vessels into which they are introduced. In contrast, in the root-injury procedure there are many points of entry provided by the cutting of large numbers of secondary roots, but the bacterial suspension is not introduced directly into the root tissues.

When relatively concentrated bacterial suspensions were used, the inoculation of 6-week-old tomato plants gave a better estimate of relative levels of resistance than when younger plants were used. If dilute bacterial suspensions were used, measurable resistance levels could be detected even in 4-week-old tomato plants. In these tests environmental conditions were extremely critical and unless optimum conditions were maintained, results at lower inoculum concentrations were not uniform. It is of interest to note that even with the standard concentration of inoculum the 2-week-old Schwarz 21 peanut plants were very highly resistant to root- or stem-inoculations.

The bacterial suspensions used in greenhouse tests were probably highly concentrated when compared with bacterial populations present under field conditions. The disease incidence in tomato plants of resistant lines grown in naturally-infested soil in the field at Oxford, N. C. in 1950 was much lower than for plants of the same lines inoculated in the greenhouse using the standard bacterial suspensions. Field resistance probably could be more closely approximated in greenhouse inoculation tests by using relatively dilute bacterial suspensions. This would also permit the testing of smaller younger plants and increase the rate at which successive experiments could be completed.

Apparently the number of tomato plants grown per flat, at least within the range of 45 to 450, has little influence on results when root-inoculations are used. Because of plant competition, however, the use of more than 50 to 100 tomato plants per flat would not be practical if plants surviving inoculation are to be transplanted directly from the flat to the field.

During the progress of these studies there was ample opportunity to observe the importance of maintaining optimum environmental conditions for disease development in order to make a proper evaluation of resistance. In particular, failure to maintain the high temperatures requisite for rapid growth of the pathogen was the most important single factor which could be associated with erratic results.

## SUMMARY

An evaluation was made of the relative effectiveness of various procedures for inoculating resistant and susceptible tomato plants with pure cultures of *P. solanacearum*. Potato, tobacco, and peanut plants were also used in certain phases of the investigation. Preliminary tests with naturally infested soil or diseased plant debris as sources of inoculum indicated the superiority of pure cultures for inoculations under greenhouse conditions.

Inoculations either by puncturing the stem through a drop of bacterial suspension placed in a leaf axil or by pouring a bacterial suspension over wounded secondary roots were equally effective on succulent seed-

lings of susceptible hosts such as Marglobe tomato, Irish Cobbler potato, and Variety 402 tobacco. In resistant tomato (L1 and L2; R2, R4 and R5), tobacco (Oxford 26, Dixie Bright 27, 101 and 102), and peanut (Schwarz 21) plants, disease indices obtained by stem-inoculations were higher than those obtained by root-inoculations. However, best differentiation between resistant and susceptible plants was made on the basis of root-inoculations.

Susceptibility to bacterial wilt decreased in resistant tomato plants as age of plants increased from 4 to 8 weeks. In the susceptible Marglobe and Louisiana Pink varieties there was no decrease in susceptibility with increase in age as long as conditions favoring rapid succulent growth were maintained.

The number of Marglobe tomato seedlings per flat was increased from 45 to 450 without significantly impairing the efficacy of the root-inoculation technique.

Decreasing the inoculum concentration by successive dilutions to 1:128 did not result in a decrease in disease severity in 4-week-old Marglobe seedlings. However, lower disease readings were obtained in resistant tomato plants (line L1) with the more dilute bacterial suspensions.

The causal bacterium was isolated from resistant tomato, tobacco and peanut plants which showed no external symptoms of the disease.

PLANT PATHOLOGY
NORTH CAROLINA STATE COLLEGE,
RALEIGH, NORTH CAROLINA

LITERATURE CITED

1. GALLEGLY, M. E. AND J. C. WALKER. 1949. Relation of environmental factors to bacterial wilt of tomatoes. Phytopathology **39**: 936-946.
2. GRIEVE, B. J. 1943. Studies in the physiology of host-parasite relations. III. Factors affecting resistance to bacterial wilt of Solanaceae. Proc. Roy. Soc. Victoria **55**: 13-40.
3. HUTCHINSON, C. M. 1913. Rangpur tobacco wilt. Memoirs Dept. Agr. India, Pusa Agr. Res. Inst., Bact. Ser. **1**: 67-83.
4. KELMAN, A. 1952. Evaluation of resistance to Pseudomonas solanacearum in tobacco under greenhouse conditions. (Abs.) Phytopathology **42**: 284.
5. KELMAN, A. AND J. H. JENSEN. 1951. Maintaining virulence in isolates of Pseudomonas solanacearum. Phytopathology **41**: 185-187.
6. MOORE, E. L., G. B. LUCAS AND E. E. CLAYTON. 1952. Granville wilt and black shank resistant tobacco varieties, N. C. Agr. Exp. Sta. Bul. 378. 18 pp.
7. NAKATA, K. 1927. Concerning the vitality and pathogenicity of Bacterium solanacearum E. F. S., a cause of tobacco wilt. Jour. Sci. Agr. Soc. (Tokyo) **296**: 283-304. (Japanese with English summary).
8. NOLLA, J. A. B. 1931. Studies on the bacterial wilt of the Solanaceae in Porto Rico. Jour. Dept. Agr. Puerto Rico **15**: 287-308.
9. ROQUE, A. 1933. Bacterial wilt of tobacco in Puerto Rico and its intertransmission to other Solanaceous hosts. Jour. Dept. Agr. Puerto Rico **17**: 145-156.
10. SCHWARZ, M. B. AND C. HARTLEY. 1950. De selectie van Schwarz 21, een tegen slijmziekte resistente lijn van Arachis hypogaea. Landbouw [Bogor] **22**: 223-244. (English summary).
11. SMITH, E. F. 1914. Bacteria in relation to plant diseases. Carnegie Inst. Wash. Vol. 3. 309 pp. (170-172; 174-271; 282).
12. SMITH, T. E. 1939. Host range studies with Bacterium solanacearum. Jour. Agr. Res. [U. S.] **59**: 429-440.
13. STANFORD, E. E. AND F. A. WOLF. 1917. Studies on Bacterium solanacearum. Phytopathology **7**: 155-165.
14. VAUGHAN, E. K. 1944. Bacterial wilt of tomato caused by Phytomonas solanacearum. Phytopathology **34**: 443-458.
15. WINSTEAD, N. N. 1952. Evaluation of resistance in tomato to Pseudomonas solanacearum by artificial inoculation methods. (Abs.) Phytopathology **42**: 23.

## PHYTOPATHOLOGICAL NOTES

***Ephelis on Echinochloa Crus-Galli Beauv. and Setaria Italica Beauv.*** N. S. VENKATAKRISHNIAH. In the course of studies of *Ephelis* on rice, observations were made of its occurrence on other hosts. The writer[1] reported the fungus on *Isachne elegans* and *Eragrostis tenuifolia.*

*Echinochloa crus-galli* Beauv., a common grass in wet lands, was observed to be infected at the Rice Breeding Station, Nagenahally, Mysore in November, 1947, and at the Visveswariah Canal Farm, Mandya, in December 1949. The infection occurred on a few plants in the midst of the rice crop. The inflorescence was greatly reduced in size and all the spikelets were glued to one another and to the central rachis. (Fig. 1, Panel 1B). The mycelium invaded young flowers and prevented their further development. The fungus formed grayish-white, sclerotioid masses which later turned black. The conidia were hyaline, needle-shaped, and measured 17.5 to 27.5 $\mu$ by 1.5 to 2 $\mu$.

The fungus on *Setaria italica* Beauv., a food grain crop, was noticed in the dry land at the Government Central Farm, Hebbal, Bangalore, in October, 1948. Infection was severe on the plants and occurred in patches in the field. Plants were greatly stunted in growth. The normal inflorescence is 3½-7 in. in length and ½-¾ in. in diameter whereas the infected one was a stiff spike ½-3 in. long and reduced in severe cases to 1/20-1/10 in. in diameter (Fig. 1, Panel 2B). Individual flowers at the lower end of the panicle were affected. In severe cases the entire inflorescence was involved. The dwarfed spikelets remained glued together to the central rachis. The fungus formed thick, gray to black sclerotioid masses. Conidia are needle-shaped, hyaline, slightly curved and possess prominent vacuoles. They measure 14-26.5 $\mu$ by 1.5-2 $\mu$.

[1] Venkatakrishniah, N. S. 1946. *Ephelis* on two new hosts. Current Sci. 15: 260-261.

The writer is thankful to Sri. S. V. Venkatarayan, Plant Pathologist, for helpful criticisms in the course of this work.

FIG. 1. Panel 1, inflorescences of Echinochloa crus-galli. Panel 2, Setaria italica: A) Healthy inflorescence compared with B) Infected inflorescence showing acute symptoms.

Tai and Siang[2] report the occurrence of *Ephelis oryzae* Syd. on *Echinochloa crus-galli* in rice fields at Kunming. There appears to be no record of its occurrence on this host or on *Setaria italica* in India. Symptoms of the infection on these 2 grasses, and the shape and size of the conidia, closely resemble those of the fungus on rice. Conidia of *Ephelis oryzae* occurring on rice measure 17.5-28 $\mu$ by 1.75 $\mu$. This differs from the measurements recorded by Sydow, viz. 20-35 $\mu$ by 1 $\mu$. Pending observation of the perfect stage, the pathogen on the 2 hosts is referred to *Ephelis oryzae*.—Plant Pathology Section, Agricultural Department, Bangalore, India.

*Tilletia elymi, an Apparently Homothallic Species.*[1] C. S. HOLTON.[2] Heterothallism in certain species of *Tilletia* has been demonstrated experimentally by pathogenicity tests[3] and the probable heterothallic nature of certain other species of this genus is indicated by the occurrence of fused pairs of primary sporidia on the promycelia. Thus far no species of *Tilletia* has been described as being homothallic. Spore germination studies with *Tilletia elymi* Diet. & Holw. from *Elymus glaucus* Buckl. in which there is evidence of homothallism are reported here.

Chlamydospores of *Tilletia elymi* were placed on water agar (2-3 per cent) in Petri dishes and incubated at 5° C. until germination occurred. Some spores began to germinate in about 1 month and additional spores continued to germinate over a period of several weeks. The amount of germination was estimated at less than 10 per cent. Several similar tests gave uniformly comparable results.

[2] Tai, F. L. and W. N. Siang 'I-chu-hsiang' disease of rice caused by *Ephelis oryzae* Sydow in Yunnan. 1948. Acta Agric. 1948 1: 125-131. Abstracted 1949 in Rev. Appl. Myc. 28: 29.

[1] Cooperative investigations between the U. S. Department of Agriculture, Agricultural Research Administration, Bureau of Plant Industry, Soils, and Agric. Engineering, Field Crops Section, Division of Cereal Crops and Diseases, and the Washington Agricultural Experiment Stations, Pullman, Washington.

[2] Pathologist, Division of Cereal Crops and Diseases.

[3] Flor, H. H. Heterothallism and hybridization in *Tilletia tritici* and *T. levis*. Jour. Agr. Res. 44: 49-58. 1932.

The promycelia were variable in length. Counts of the primary sporidia on 34 promycelia showed 14 with 5, 16 with 4, and 4 with 3 sporidia. Such comparably small numbers of primary sporidia have not been reported for known heterothallic species of *Tilletia*.

The primary sporidia of *Tilletia elymi* have a relatively large diameter (2.5-3$\mu$) and a wide range in length (60-85$\mu$). The protoplasmic content appears to be highly turgid. This is suggested by the rigid character of the sporidia, which will "bend" under stress but "spring back" to the original position when freed. These characteristics are in contrast to those of heterothallic species, in which the sporidia are relatively slender and highly flexible.

Growth of the primary sporidia in culture, whether on water agar or on a nutrient medium, begins with the production of a germ tube into which the protoplasm migrates, leaving the empty sporidium behind. As this tube elongates, cell walls are formed at intervals at the base of the protoplasm, which remains concentrated in the relatively long apical cell. No branching of any kind occurs. Growth in this manner continues indefinitely, resulting in a long, empty, cellular strand attached to the protoplasmic apical cell. The germ tubes of fused pairs of primary sporidia of the heterothallic species *Tilletia caries* (DC.) Tul. and *T. foetida* (Wallr.) Liro developed in a similar manner.[4]

Many primary sporidia of *Tilletia elymi* were isolated and only 1 developed a mycelial colony in culture. This colony grew slowly, was leathery and tough, unsuitable for inoculation purposes. Relatively large sickle-shaped, secondary sporidia were formed occasionally on the agar where spores were germinating. Growth was observed in a few secondary sporidia, similar to that in primary sporidia.

Primary and secondary sporidia were stained with iron-alum-haematoxylin and were found to be multinucleate (Figure 1). Of 61 primary sporidia observed 1 had 2 nuclei, 13 had 3, 15 had 4, 25 had 5, and 7 had 6 nuclei. Observations on 8 secondary sporidia showed that 4 of them had 3 nuclei, 3 had 4, and 1 had 5 nuclei. This multi-nucleate condition of the sporidia probably is related to their apparent homothallic nature.

FIG. 1. Camera lucida drawings of 3 primary and 2 secondary sporidia of *Tilletia elymi* illustrating their multinucleate character (× approx. 1200). (Illustration prepared by John Y. Woo.)

Apparent homothallism of *Tilletia elymi* is indicated, therefore, by the relatively small number of primary sporidia, their morphological characters, the absence of fusions between them, the nature of their growth in culture, and the nuclear condition of both primary and secondary sporidia. Positive evidence from pathogenicity tests has not been obtained.—Washington Agricultural Experiment Station, Pullman, Washington.

[4] Holton, C. S. and Heald, F. D. Bunt or Stinking Smut of Wheat (a world problem). 211 pp. Burgess Publishing Co., Minneapolis, Minn. 1941.